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Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease (Review)

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[Intervention Review]

Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

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ABSTRACT

Background

Cystic fibrosis is caused by a defective gene encoding a protein called the cystic fibrosis transmembrane conductance regulator (CFTR), and is characterised by chronic lung infection resulting in inflammation and progressive lung damage that results in a reduced life expectancy.

Objectives

To determine whether topical CFTR gene replacement therapy to the lungs in people with cystic fibrosis is associated with improvements in clinical outcomes, and to assess any adverse effects.

Search methods

We searched the Cochrane Cystic Fibrosis and Genetic Disorders Group Trials Register comprising references identified from comprehensive electronic database searches, handsearching relevant journals and abstract books of conference proceedings.

Date of most recent search: 05 May 2016.

An additional search of the National Institutes for Health (NIH) Genetic Modification Clinical Research Information System (GeMCRIS) was also performed for the years 1992 to 2015.

Date of most recent search: 20 April 2016.

Selection criteria

Randomised controlled studies comparing topical CFTR gene delivery to the lung, using either viral or non-viral delivery systems, with placebo or an alternative delivery system in people with confirmed cystic fibrosis.

Data collection and analysis

The authors independently extracted data and assessed study quality. Authors of included studies were contacted and asked for any available additional data. Meta-analysis was limited due to differing study designs.

Main results

Four randomised controlled studies met the inclusion criteria for this review, involving a total of 302 participants lasting from 29 days to 13 months; 14 studies were excluded. The included studies differed in terms of CFTR gene replacement agent and study design, which limited the meta-analysis. One study only enrolled adult males, the remaining studies included both males and females aged 12 years and over.

Risk of bias in the studies was moderate. Random sequence generation and allocation concealment was only described in the more recent study; the remaining three studies were judged to have an unclear risk of bias. All four studies documented double-blinding to the intervention, but there is some uncertainty with regards to participant blinding in one study. Some outcome data were missing from all four studies.

There were no differences in either the number of respiratory exacerbations or the number of participants with an exacerbation between replacement therapy or placebo groups at any time point. Meta-analysis of most respiratory function tests showed no difference between treatment and placebo groups, but the smallest study (n = 16) reported forced vital capacity (litres) increased more in the placebo group at up to 24 hours. A further study reported a significant improvement in forced expiratory volume at one second (litres) at 30 days after participants had received their first dose of favouring the gene therapy agent, but this finding was not confirmed when combined with at second study in the meta-analysis. The more recent study (n = 140) demonstrated a small improvement in forced vital capacity (per cent predicted) at two and three months and again at 11 and 12 months for participants receiving CFTR gene replacement therapy compared to those receiving placebo. The same study reported a significant difference in the relative change in forced expiratory volume at one second (per cent predicted) at two months, three months and 12 months.

One small study reported significant concerns with "influenza-like" symptoms in participants treated with CFTR gene replacement therapy; this was not reported on repeated use of the same agent in a larger recent study.

There was no other evidence of positive impact on outcomes, in particular improved quality of life or reduced treatment burden.

Two studies measured ion transport in the lower airways; one (n = 16) demonstrated significant changes toward normal values in the participants who received gene transfer agents ($P < 0.0001$), mean difference 6.86 (95% confidence interval 3.77 to 9.95). The second study (n = 140) also reported significant changes toward normal values ($P = 0.032$); however, aggregate data were not available for analysis. In the most recent study, there was also evidence of increased salt transport in cells obtained by brushing the lower airway. These outcomes, whilst important, are not of direct clinical relevance.

Authors' conclusions

One study of liposome-based CFTR gene transfer therapy demonstrated some improvements in respiratory function in people with CF, but this limited evidence of efficacy does not support this treatment as a routine therapy at present. There was no evidence of efficacy for viral-mediated gene delivery.

Future studies need to investigate clinically important outcome measures.

PLAIN LANGUAGE SUMMARY

Replacing the defective gene is a potential treatment for progressive lung disease in people with cystic fibrosis

Review question

We reviewed the evidence about the effect of delivering the correct copy of the cystic fibrosis transmembrane conductance regulator (CFTR) gene directly to the lungs of people with cystic fibrosis in order to treat progressive lung disease.

Background

In cystic fibrosis the gene encoding a protein called the cystic fibrosis transmembrane conductance regulator (CFTR) is faulty. People with cystic fibrosis suffer from progressive lung infection and damage which reduces life expectancy. Agents which can deliver a correct copy of the faulty CFTR gene to cells in the lungs may be an effective treatment.

Search date

The evidence is current to: 20 April 2016.

Study characteristics

We found four studies with 302 people to include in this review. The studies lasted from 29 days to 13 months. Three of these studies included both men and women aged 12 years and over and one study only included adult men. The studies compare gene therapy to a dummy treatment (placebo) both of which are inhaled as a mist into the lungs. The studies were of different designs and used different agents. This meant we could not combine their results.

Key results

Three of the studies, including the largest and most recent study, showed an improvement in some measures of lung function in people with CF given gene therapy. We did not find that any more clinically relevant outcomes such as quality of life, treatment burden or flare-up of lung disease had improved with treatment. In one study "influenza-like" symptoms were more common in people who received CFTR gene transfer agents but this was not reported when the agent was used repeatedly in a larger study. In those people who took the gene transfer agents, molecules and salt in their lower airways moved more like they do in healthy people.

The limited evidence of benefit does not support this as a routine therapy at present. We recommend that future studies are designed and reported clearly so that their results can be incorporated into a systematic review.

Quality of the evidence

The most recent study provided detailed information on how the people were put into different treatment groups completely at random, and so we are satisfied that those taking part in the study had an equal chance of being in either group (CFTR gene transfer agent or placebo) and that no one could work out which group the next person would be put into. The other studies reported that people were put into groups at random but did not specify how, so we cannot be sure that there was an equal chance of them being in either group. We believe that the clinicians running all the studies did not know which treatment the people taking part were receiving and that in three of the studies those taking part did not know either, but we could not be sure whether the people taking part in the latest study knew which treatment they were receiving and what effect this knowledge might have on results. Unfortunately, the studies did not report all their results clearly; sometimes results were not reported in a way that we could use for the review and sometimes they were not reported at all. This reduced the certainty with which we judged the overall results.

BACKGROUND

Description of the condition

Cystic fibrosis (CF) is the commonest life-shortening disease in Caucasians and is caused by a single gene defect. It has a prevalence of approximately 1 in 2000 at birth (Bobadilla 2002). The affected gene is responsible for making a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). There are many abnormalities or alterations of the CFTR gene that can affect the production and function of CFTR (Rowntree 2003). Normally, CFTR has an important role in co-ordinating salt transport across cell membranes. This role is particularly important in the lungs where an abnormality of CFTR results in dehydration of the surface liquid that lines the airways (Matsui 1998). Consequently the airway is not able to remove bacteria and chronic airway infection results in an intense local inflammation and mucus secretion (Boucher 2004). Subsequently a cycle of increasing lung inflammation and lung damage progressively reduces lung function, leading eventually to premature death (often in the third or fourth decade of life) from respiratory failure (FitzSimmons 1993; Frederiksen 1996).

Description of the intervention

These disease processes could be prevented by inserting a correct copy of the CFTR gene into the cells of people with CF, a process termed CFTR gene transfer therapy (Griesenbach 2004).

How the intervention might work

There are three main reasons why CFTR gene transfer therapy has been proposed for CF lung disease:

1. the CFTR gene has been identified and characterised and it is possible to manufacture artificial CFTR genes (Riordan 1989);

2. CF is a progressive condition with a potential window for early treatment when the lungs are relatively unaffected (Ranganathan 2004);

3. delivery of gene therapy reagents to the lungs may be possible by aerosolisation or other methods of topical application (Lee 2005).

Early laboratory studies demonstrated the successful transfer of the CFTR gene to cells and animal airways using a variety of different gene delivery methods (Southern 1996). Essentially, delivery agents have been described as viral (where the CFTR gene is incorporated into a replication incompetent virus) and non-viral (most commonly positively-charged liposomes which when mixed with DNA increases uptake into cells) (Lee 2005). Human studies using viral vectors suggest that topical delivery to nasal airway cells results in uptake of the CFTR gene and expression of the gene by the airway cells (Knowles 1995; Zabner 1996). These studies demonstrate some correction of abnormal salt transport; however, repeat dosing results in inflammation (immune-mediated) and no detectable gene transfer (Harvey 1999; Zabner 1996). Repeated dosing of non-viral gene delivery agents does not result in such marked immune-mediated responses; however, steady-state levels of gene expression and functional changes have been less impressive than those achievable acutely with viral vectors (Hyde 2000). Much effort is being directed towards developing gene transfer agents and strategies with both improved gene uptake and expression, and reduced immunogenicity (Ferrari 2002; Griesenbach 2004; Lee 2005).

Why it is important to do this review

This review is an updated version of previous Cochrane Reviews (Lee 2007; Lee 2012). It focuses on treatment of CF lung disease as this is the major cause of morbidity and mortality in people with CF, however abnormal CFTR function affects other parts of the body, some of which may be amenable to other forms of gene transfer therapy but will not be the subject of this review.

OBJECTIVES

To investigate whether topical CFTR gene replacement therapy to the lungs of people with CF is associated with improvements in clinical outcomes (respiratory function and quality of life) and assess existing or predicted adverse effects.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs), published or unpublished. Controlled clinical trials (CCTs), including quasi-randomised controlled trials, will be included in future updates if available and only if there is sufficient evidence that control and intervention groups were similar at baseline.

Types of participants

Children and adults with CF confirmed by the presence of two disease-causing mutations, or by a combination of positive sweat test and recognised clinical features of CF.

Types of interventions

Topical CFTR gene delivery to the lung, using either viral or non-viral vector systems, compared to placebo or an alternative vector system.

Types of outcome measures

Primary outcomes

1. Respiratory exacerbations (assessed by need for additional oral or intravenous antibiotics)*
   - i) oral antibiotics (days or episodes)
   - ii) intravenous antibiotics (days or episodes)
2. Lung function testing (absolute values or percent predicted for age, sex and height)
   - i) forced expiratory volume at one second (FEV_1)
   - ii) forced vital capacity (FVC)
   - iii) relative change in FEV_1 or FVC
   - iv) other relevant lung function tests (for example, infant lung function tests and lung clearance index)
3. Survival (either as a binary outcome or time to event)*

Secondary outcomes

1. Number of days as a hospital inpatient (or number of inpatient episodes)*
2. Need for extra treatment*
   - i) physiotherapy (duration or episodes)
3. Adverse events*
   - i) mild (e.g. sore throat, hoarse voice, dry mouth)
   - ii) moderate (e.g. wheeze, cough, fever, local allergic reaction)
   - iii) severe (e.g. coughing up blood (haemoptysis), collapsed lung (pneumothorax), chest infection, systemic allergic reactions)
4. Quality of life (as measured by a validated and appropriate method)*

5. School or work attendance*
6. Nutritional parameters (including z scores or centiles)
    i) weight
    ii) body mass index (BMI)
    iii) height
7. Acquisition of newly cultured respiratory pathogens
    i) *Pseudomonas aeruginosa*
    ii) *Staphylococcus aureus*
    iii) *Haemophilus influenzae*
8. Sputum rheology (stickiness, as measured by a validated method)
9. Mucociliary clearance of the airway (as measured by a validated method)
10. Airway potential difference measurements (measuring salt transport)
    i) Baseline potential difference
    ii) Response to perfusion with amiloride
    iii) Response to perfusion with a zero chloride solution
11. Measures of gene expression
    i) quantitative reverse transcription polymerase chain reaction (rtPCR) to measure vector-specific CFTR messenger ribonucleic acid (mRNA) production
    ii) any other method of measuring gene expression
12. Measures of CFTR protein expression
13. Radiological measures of lung disease
    i) validated chest radiograph scores
    ii) validated computerised tomogram (CT) score

*indicates outcomes that have a direct influence on the person with CF (and therefore are more pragmatic measures of efficacy).

Search methods for identification of studies

Electronic searches

Relevant studies were identified from the Group's Cystic Fibrosis Trials Register using the search term: topical (aerosolized) CFTR gene replacement for the lungs.

The Cystic Fibrosis Trials Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of *The Cochrane Library*), quarterly searches of MEDLINE, a search of Embase to 1995 and the prospective handsearching of two journals - *Pediatric Pulmonology* and the *Journal of Cystic Fibrosis*. Unpublished work is identified by searching the abstract books of three major cystic fibrosis conferences: the International Cystic Fibrosis Conference; the European Cystic Fibrosis Conference and the North American Cystic Fibrosis Conference. For full details of all searching activities for the register, please see the relevant sections of the Cystic Fibrosis and Genetic Disorders Group Module.

Date of latest search of the Group's Trials Register: 05 May 2016.

An additional search of the National Institutes for Health (NIH) Genetic Modification Clinical Research Information System (GeMCRIS) was also performed for the years 1992 to 2016 (Appendix 1).

Date of latest search: 20 April 2016.

Searching other resources

Investigators who work in the field and previous authors were also contacted for unpublished or additional data.

Data collection and analysis

Selection of studies

For the initial review and earlier updates, two authors (TWRL and KWS) independently selected the studies to be included in the review. From 2015 two co-authors (JP-D and LP) selected studies. There was agreement between the authors on the suitability of studies for inclusion in the review.

Data extraction and management

For the initial review and earlier updates, two authors (TWRL and KWS) independently extracted data for the review. From 2015 two co-authors (JP-D and LP) extracted data from the included studies. The authors used a standardised form to extract data from studies eligible for inclusion in the review. If the publications provided standard errors (SEs), the authors converted these to standard deviations (SDs). The authors undertook meta-analysis on data from all included studies.

As the effect of gene transfer therapy may be cumulative, we analysed studies with different durations of intervention separately. For example, we planned to combine studies of less than one month duration, studies between one and two months duration, three and four months duration and five and six months duration. We planned to combine studies longer than six months duration on a three-monthly basis. Regarding studies of less than one month duration, in a *post hoc* change to protocol, we did not consider it appropriate to combine six-hour data with Day 30 data, as data obtained within 24 hours of administration are related to monitoring of adverse effects rather than efficacy.

We report when measurements were taken by the primary investigators during the study, what measurements were reported within the published paper and what data we are reporting in the review (*see* Additional Tables (Table 1; Table 2)).

Assessment of risk of bias in included studies

In order to establish a risk of bias, two authors independently assessed the studies to be included in the review. Originally, TWRL

and KWS assessed the methodological quality of each study based on a method described by Jüni and colleagues (Jüni 2001). The authors assessed the following:

- a description of the degree of blinding;
- inclusion of all participants in an intention-to-treat analysis, regardless of whether they completed the treatment schedule or not;
- whether there is a clear description of generation of allocation sequence, for example with a random numbers table;
- whether concealment of allocation sequence is described, for example if neither investigators nor participants can foresee assignment to either treatment or control group.

There was agreement between the authors on the quality of studies for inclusion in the review.
For more recent updates of the review, all authors assessed the risk of bias of the included studies using the risk of bias tool developed by Cochrane (Higgins 2011).

Measures of treatment effect

For binary outcome measures, the authors calculated a pooled estimate of the treatment effect for each outcome across studies using the risk ratio (RR). For continuous outcomes, they recorded either mean relative change from baseline for each group or mean post-treatment or intervention values and SD. They calculated a pooled estimate of treatment effect by calculating the mean difference (MD) with 95% confidence intervals (CIs). Authors converted any SEs to SDs. To calculate the SD for change from baseline in bronchial potential difference from the most recent Alton study, authors assumed the correlation coefficient to be 0.9 and performed sensitivity analysis at a range of values (Alton 2015). For studies reporting continuous outcomes at multiple time points there are currently no methods to analyse aggregate longitudinal data if individual patient data are not available. Therefore in this review the authors have carried out analysis at each individual time point reported and assumed zero correlation between results. For any outcomes producing time-to-event data (e.g. survival) or count data (e.g. number of days), they analysed the data using the most appropriate method.

Unit of analysis issues

The review authors planned to consider studies with a cross-over design for inclusion; however, they have concerns about the potential for 'hangover effects' following too brief a washout period (Southern 2003). This is particularly pertinent for gene transfer therapy agents which may alter the natural history of the CF airway disease. The review authors would have analysed any data from cross-over studies that were eligible for inclusion using a method described by Elbourne (Elbourne 2002). Elbourne says that this approach will produce conservative results, as it does not take into account within-patient correlation. Also, each participant will appear in both the treatment and control group, so the two groups will not be independent. If cross-over studies are included in future updates of this review we will undertake a sensitivity analysis to determine the effect of these studies on the overall result.

Dealing with missing data

In order to allow an intention-to-treat (ITT) analysis, the review authors sought data on the number of participants with each outcome event, by allocated treated group, irrespective of compliance and whether or not the individual was later thought to be ineligible or otherwise excluded from treatment or follow up. The review authors contacted the investigators of one study and obtained some additional data for inclusion in the review (Alton 2015).

Assessment of heterogeneity

Since the authors only included four studies and were not able to combine data for many outcomes, they did not assess heterogeneity. If they are able to include more studies in future updates of the review, they will assess heterogeneity through a visual examination of the combined data presented in the forest plots, and by considering the I^2 statistic (Higgins 2003) together with Chi ^{2} values and their CIs (Deeks 2011). This reflects the likelihood that variation of results across trials are due to heterogeneity rather than chance, and the authors will interpret this statistic using the following simple classification:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

Assessment of reporting biases

In order to identify selective outcome reporting, where possible, the authors compared outcomes defined in the protocol with those reported in the full publication. The authors requested the protocol for the study from the primary investigators, corresponding author(s), or relevant pharmaceutical company and recorded whether the protocol was available or not. They also compared outcomes listed in the 'Methods' section of the final paper with those presented in the 'Results' section. For negative findings that were reported either only partially, or not at all, the authors contacted primary investigators for these data.
In future updates, should sufficient studies become available, the authors will attempt to assess whether this review is subject to publication bias by using a funnel plot (which graphically illustrates variability between studies and should not demonstrate a systematic difference). If the authors detect asymmetry, they will explore causes other than publication bias.

Data synthesis

The authors have analysed the data included in the review using a fixed-effect model. If in future they identify a moderate or high degree of heterogeneity, they plan to analyse the data using a random-effects model.

Subgroup analysis and investigation of heterogeneity

If the authors had identified any heterogeneity, they would have investigated this using subgroup analysis of potential effect modifiers. The major potential effect modifier is age, other effects include sex and treatment centre.

Sensitivity analysis

If cross-over studies are included in future updates of this review the authors will undertake a sensitivity analysis to determine the effect of these studies on the overall result.

RESULTS

Description of studies

Results of the search

The searches identified 18 studies in the searches, but only four were eligible for inclusion in the review; the remaining 14 studies were excluded from the review.

Included studies

Four of the 18 studies identified in the searches were eligible for inclusion in the review (Alton 1999; Alton 2015; Moss 2004; Moss 2007).

Study Design

All four studies were RCTs of parallel design comparing CFTR gene replacement delivered to the lungs of people with CF to placebo (Alton 1999; Alton 2015; Moss 2004; Moss 2007). One study was single centre (Alton 1999) and the remaining studies were multicentre; one was conducted at two sites recruiting from 18 centres (Alton 2015), one recruited from eight centres (Moss 2004) and the remaining one from 12 centres (Moss 2007). The Moss studies were conducted in the USA and the Alton studies were conducted in the UK (Alton 1999; Alton 2015; Moss 2004; Moss 2007).

The largest study randomised 140 participants into treatment and placebo groups and 136 participants received at least one dose of the study drug (Alton 2015). The second largest study recruited 109 participants and 102 received at least one dose of the study drug (Moss 2007). Moss 2004 randomised 42 participants and 37 received at least one dose of study drug (Moss 2004). The smallest study randomised 16 participants and all received at least one study dose (Alton 1999).

Three studies reported power calculations (Alton 2015; Moss 2004; Moss 2007). Alton calculated adequate power to test differences between treatment groups for the primary protocol endpoint, relative change in % predicted FEV_1 (Alton 2015). Both Moss studies reported calculating adequate power to test differences between treatment groups with respect to the primary and secondary protocol end points (Moss 2004; Moss 2007).

The longest follow up of participants was 13 months (Alton 2015) and shortest was 29 days (Alton 1999). In Moss 2004, follow up was 150 days (Moss 2004), whereas Moss 2007 had outcome-dependent follow up, with 90 days for lung function and 210 days for adverse event monitoring (Moss 2007).

Participants

In total, all four studies randomised 302 participants with CF (Alton 1999; Alton 2015; Moss 2004; Moss 2007). One study recruited only male participants due to regulatory restrictions (Alton 1999). The remaining three studies recruited both males and females with an approximately even gender split in each group, except for the treatment group in Moss 2004 where there were more than double the number of females to males (14 females, six males) (Alton 2015; Moss 2004; Moss 2007).

With regards to age, one study specified that all participants were adults with a mean age of 26.9 years (Alton 1999). The remaining three studies recruited participants of at least 12 years of age; the mean age of participants was 24.7 years (Alton 2015), 23.7 years (Moss 2004) and 22.6 years (Moss 2007). A breakdown of the mean ages of participant by treatment group is available in the tables (Characteristics of included studies).

All studies reported the genotypes of participants. Alton 1999 reported that 12 out of 16 participants were homozygous ΔF508, but did not report the numbers for each mutation separately by treatment group (Alton 1999). Alton 2015 stated that the homozygous ΔF508 genotype was the commonest, with 48% in the placebo group and 50% in the treatment group (Alton 2015). In Moss 2004 there were significantly more ΔF508 homozygous participants (77%) in the placebo group than in the CFTR gene replacement group (25%) (P = 0.01) (Moss 2004). Moss 2007 recruited slightly more ΔF508 homozygous participants - 53% in each group (Moss 2007).

With regard to other participant characteristics, all studies stated a minimum FEV_1 % predicted score; at least 70% (Alton 1999), at least 50% (Alton 2015) and at least 60% (Moss 2004; Moss 2007). Alton 1999 reported that there were no significant differences between groups in terms of BMI, or chest x-ray score (Alton

1999) and Alton 2015 reported no significant difference in BMI and centre distribution number (Alton 2015). Both Moss studies reported that treatment and placebo groups were similar in terms of demographics and clinical characteristics (Moss 2004; Moss 2007).

Interventions

All four studies compared an active treatment to a placebo (Alton 1999; Alton 2015; Moss 2004; Moss 2007). The Alton studies delivered the CFTR gene as plasmid DNA complexed with liposome (GL-67/DOPE/DMPE-PEG_{5000}). In the treatment group for the 1999 study, Alton nebulised 16 ml from a 20 ml solution containing 42.2 mg of plasmid DNA (pCF-1-CFTR) once to the lungs whilst the placebo group received liposome alone (Alton 1999). In Alton 2015, the treatment group received 5 ml nebulised solution containing 13.3 mg of plasmid DNA, pGM169, at 28-day intervals (plus or minus five days) over 12 months, whilst the placebo group received 5 ml of nebulised 0.9% saline (Alton 2015).

Both Moss studies used the adeno-associated virus serotype 2 (AAV-2) vector tgAAVCF to deliver the CFTR gene (Moss 2004; Moss 2007). In Moss 2004, $1x10^{13}$ particles tgAAVCF or matching placebo were nebulised to the lungs on three occasions 30 days apart (Moss 2004). Moss 2007 used a similar design, but used two instead of three doses, but still 30 days apart (Moss 2007).

Outcomes

Adverse events and lung function were the only outcomes measured by all four trials (Alton 1999; Alton 2015; Moss 2004; Moss 2007). However, lung function was reported in a number of ways: three studies reported on the change from baseline in FEV_1 (L) (Alton 1999; Moss 2004; Moss 2007); two studies reported the change from baseline in FEV_1 (% predicted) (Moss 2004; Moss 2007); three studies reported on the change from baseline in FVC (L) (Alton 1999; Moss 2004; Moss 2007); and one study reported on both the change from baseline in FVC (% predicted) and the relative change in FEV_1 (% predicted) (Alton 2015). Three studies reported on respiratory exacerbations (Alton 2015; Moss 2004; Moss 2007). Both Alton studies and Moss 2004 reported on changes in measures of CFTR protein function (gene expression, CFTR protein expression and airway potential difference) (Alton 1999, Alton 2015; Moss 2004). Two studies reported on computerised tomography (Alton 2015; Moss 2004). Alton 2015 additionally reported on quality of life (QoL) assessment, lung clearance index, and changes in sputum microbiology (Alton 2015). Moss 2004 reported on inpatient episodes and the acquisition of new pathogens (Moss 2004).

Excluded studies

We excluded 14 studies from the results of the search (*see* Characteristics of excluded studies). Eight of the excluded studies were not RCTs (Davies 2011; Flotte 1996; Harvey 1999; Joseph 2001; Noone 2000; Zabner 1996; Zabner 1997; Zuckerman 1999). The remaining six studies were excluded since the therapy was not applied directly to the lungs; four studies applied treatment to the nose (Gill 1997; Hyde 2000; Knowles 1995; Porteous 1997) and two studies applied treatment to the sinuses (Wagner 1999; Wagner 2002).

Risk of bias in included studies

Allocation

All four included studies were described as randomised (Alton 1999; Alton 2015; Moss 2004; Moss 2007), but only one gave any specific details of the methodology for randomisation and concealment of allocation (Alton 2015). We therefore judged Alton 2015 to have a low risk of bias regarding the generation of allocation sequence and the concealment of allocation sequence, and the remaining three studies to have an unclear risk of bias (Alton 1999; Alton 2015; Moss 2004; Moss 2007).

Blinding

In all four studies there was double blinding to the intervention (Alton 1999; Alton 2015; Moss 2004; Moss 2007); however, only the Alton studies explicitly stated that both participants and outcome assessors were blinded (Alton 1999; Alton 2015). Alton 2015 used 0.9% saline as the placebo; and although the investigators stated that nebulisers were sealed we cannot be sure that participants would have been adequately blinded to the study drug due to potential differences in taste and consistency (Alton 2015). We therefore deemed all four studies to have a low risk of bias with regards to blinding of personnel (Alton 1999; Alton 2015; Moss 2004; Moss 2007). However, we judged three studies to have a low risk of bias with regards to blinding of participants (Alton 1999; Moss 2004; Moss 2007) and one study to have an unclear risk of bias (Alton 2015).

Incomplete outcome data

Alton 1999 assessed all randomised participants on an ITT basis, although data are incomplete for four of the reported endpoints (nasal histology, viability of lipid complex, chloride efflux and bacterial adherence) (Alton 1999). For these endpoints there is thus a potential risk of bias.

Alton 2015 analysed the 136 participants (97% of total originally randomised) who received at least one dose of the study agent on an ITT basis; 116 (83%) participants received a minimum of nine doses of the study agent and comprised the per-protocol

population (Alton 2015). Eight participants receiving placebo and 16 participants from the active group withdrew from the study and reasons were given for all of these. The only endpoint reported as ITT is relative change in FEV_1 % predicted, whereas all other outcomes are analysed as per protocol (Alton 2015). For the per-protocol endpoints, there is a potential risk of bias.

Moss 2004 randomised 42 participants; 37 (88%) received at least one dose of study drug and had outcome measures reported, and 35 (83%) completed all three doses of the study agent (Moss 2004). It is not stated whether those who withdrew had been allocated the active or placebo treatment. Results were pooled for all 37 participants receiving at least one dose of study drug. Follow up was complete for these participants for most endpoints to 150 days, but only 35 participants underwent HRCT lung scans, and just 10 had vector-specific CFTR gene expression assessed, resulting in a high risk of potential bias (Moss 2004).

Moss 2007 randomised 109 participants; 102 (94%) received at least one dose of the study drug and had outcome measures reported (Moss 2007). Reasons for withdrawal or their treatment allocation were not reported for the seven participants who did not receive the study drug post randomisation. Respiratory function data were reported to 90 days for all 102 participants receiving the study drug. Four participants receiving the study drug discontinued follow up after day 90 and before day 210; one participant from the active group and three from the placebo group withdrew (reasons given). Data on induced sputum inflammatory markers were reported for 97 participants receiving the study drug (89% of those randomised) (Moss 2007). We deemed this study to have a low risk of bias from incomplete outcome data.

Selective reporting

Some secondary endpoints from two studies remain unreported, which presents the potential for a risk of bias (Alton 2015; Moss 2007). We judged the remaining two studies to have an unclear risk of reporting bias (Alton 1999; Moss 2004).

Other potential sources of bias

In Moss 2004, the placebo group had significantly more ΔF508 homozygous participants (77%) than the CFTR gene replacement group (25%) (P = 0.01), which could affect the validity of this study due to the potential for gene replacement therapy to be more effective in participants with certain CFTR gene abnormalities versus others (Moss 2004).

Effects of interventions

Primary outcomes

1. Respiratory exacerbations

Three studies reported on this outcome (Alton 2015; Moss 2004; Moss 2007). As the studies examined different time scales (150 days, 210 days and 11 to 12 months), and additionally Alton examined number of participants rather than episodes and did not differentiate between the use of oral and intravenous antibiotics, it is not possible to combine these data in a single meta-analysis.

Both Moss studies reported on the number of respiratory exacerbations requiring intravenous antibiotics (Moss 2004; Moss 2007). Neither study found a significant difference between groups, either at 150 days RR 1.70 (95% CI 0.50 to 5.79) or at 210 days RR 1.33 (95% CI 0.62 to 2.89) (Analysis 1.1). Alton reported on the number of participants receiving additional antibiotics (oral or intravenous) between 11 and 12 months; they reported no significant difference between treatment groups, MD 1.06 (95% CI 0.73 to 1.53) (Analysis 1.2) (Alton 2015).

2. Lung function testing

All four studies reported lung function testing as a measure of effectiveness but presented their results using different measures. Three studies reported at time points between 14 days and 12 months following initial dose, (Alton 2015; Moss 2004; Moss 2007). One study reported lung function at six hours post-dose to monitor for adverse effects (Alton 1999). This meant that there are limited opportunities to combine data in a meta-analysis.

a. Forced expiratory volume at one second (FEV_1)

Three studies reported results for FEV_1 (L) (Alton 1999; Moss 2004; Moss 2007).

Alton 1999 reported a fall in FEV_1 at six hours post-dose (Alton 1999). There was no significant difference between active (n = 8) and placebo (n = 8) groups, MD -1.4 L (95% CI -3.07 to 0.27) (Analysis 1.3).

Moss 2004 reported change in FEV_1 (L) from baseline at 30 days, 60 days, 90 days, and 150 days (Moss 2004). Moss 2007 reported change this at 14 days, 30 days, 45 days, 60 days, 75 days and 90 days (Moss 2007). As the participants in Moss 2004, but not Moss 2007, received a third dose of CFTR gene replacement or placebo on Day 60, meta-analysis was only possible on data from Day 30 and Day 60 compared to baseline, and not at subsequent time points. At Day 30, the earlier study demonstrated a significant improvement in favour of the gene therapy group (n = 20) compared to the placebo group (n = 17), MD 0.14 L (95% CI 0.02 to 0.26) (Moss 2004). However, at the same time point Moss 2007 did not show a significant improvement, gene therapy group (n = 51) and placebo group (n = 51), MD 0.03 (95% CI -0.05 to 0.11) (Moss 2007). Combined data for both Moss studies show no significant difference between gene therapy (n = 71) and placebo (n = 68) groups, MD 0.06 (95% CI 0.00 to 0.13) (Analysis 1.3).

At Day 60, Moss 2004 reported no significant difference between gene therapy (n = 19) and placebo (n = 17) groups on change

in FEV_1 (L) from baseline, MD 0.05 L (95% CI -0.12 to 0.22) (Moss 2004). At the same time point, Moss 2007 also showed no significant difference between treatment groups (gene therapy n = 51; placebo n = 51), MD -0.07 (95% CI -0.15 to 0.01) (Moss 2007). Combined data for gene therapy groups (n = 70) and placebo groups (n = 68) at Day 60 show no significant difference, MD -0.05 L (95% CI -0.12 to 0.02) (Analysis 1.3).

At subsequent time points (data not presented in the meta-analysis), there were no significant differences in the change in absolute FEV_1 from baseline demonstrated in either Moss 2004 (90 days and 150 days) or Moss 2007 (45 days, 60 days, 75 days and 90 days) (Moss 2004; Moss 2007); furthermore, these data cannot be pooled due to different study regimens.

The two Moss studies reported change in FEV_1 % predicted (Moss 2004; Moss 2007). At Day 30, the Moss 2004 reported no significant difference between the groups (gene therapy n = 20; placebo n = 17) in change in FEV_1 % predicted, MD 2.99% (95% CI -0.44 to 6.42) (Moss 2004). At the same time point, Moss 2007 also reported no significant difference between the groups (gene therapy n = 51; placebo n = 51), MD 0.80% (95% CI -1.50 to 3.10) (Moss 2007). Combined data for the two Moss studies at Day 30 show no significant difference between groups (gene therapy n = 71; placebo n = 68), MD 1.48% (95% CI -0.43 to 3.39) (Analysis 1.4).

At Day 60, Moss 2004 reported no significant difference between groups (gene therapy n = 19; placebo n = 17) for the change from baseline in FEV_1 % predicted, MD 0.39% (95% CI -4.31 to 5.09) (Moss 2004). At the same time point, Moss 2007 significantly favoured placebo (n = 51) over gene therapy (n = 51), MD -2.69% (95% CI -5.14 to -0.24) (Moss 2007). Combined data for these two studies at Day 60 show no significant difference between groups (gene therapy n = 70; placebo n = 68), MD -2.03% (95% CI -4.21 to 0.14) (Analysis 1.4).

At subsequent time points there were no significant differences in change in FEV_1 % predicted from baseline demonstrated in either study and the data cannot be pooled for analysis (Moss 2004; Moss 2007).

b. Forced vital capacity (FVC)

Four studies reported different measures of FVC at a range of time points (Alton 1999; Alton 2015; Moss 2004; Moss 2007).

Alton 1999 reported a fall in FVC (L) at six hours post-dose, but there was no significant difference found between the gene therapy group (n = 8) and the placebo group (n = 8), MD -1.70 L (95% CI -3.27 to -0.13) (Alton 1999).

Both Moss studies assessed the change in FVC (L) from baseline at Day 30 and Day 60 (Moss 2004; Moss 2007). At Day 30, the earlier study reported no significant difference between the gene therapy group (n = 20) and placebo group (n = 17), MD 0.13 L (95% CI -0.02 to 0.28) (Moss 2004). At this time point Moss 2007 also demonstrated no significant difference between the groups (gene therapy n = 51; placebo n = 51) MD -0.01 L (95% CI -0.09 to 0.07) (Moss 2007). Combined data for the two Moss studies at Day 30 show no significant difference between the gene therapy group (n = 71) and placebo group (n = 68), MD 0.02 L (95% CI -0.05 to 0.09) (Analysis 1.5). At Day 60, Moss 2004 reported no significant difference between the groups (gene therapy n = 19; placebo n = 17), MD 0.02 L (95% CI -0.16 to 0.20) (Moss 2004). LIkewise, Moss 2007 also reported no significant difference between the groups (gene therapy n = 51; placebo n = 51), MD -0.07 L (95% CI -0.15 to 0.01) (Moss 2007). Combined data at Day 60 show no significant difference between gene therapy (n = 70) and placebo (n = 68), MD -0.06 L (95% CI -0.13 to 0.02) (Analysis 1.5). At later time points there remained no significant difference in the change from baseline in FVC (L) when comparing CFTR gene replacement to placebo in either study; these data were not suitable for pooling in a meta-analysis.

Alton 2015 reported the change in FVC % predicted from baseline at monthly intervals up to 12 months (Alton 2015). Alton demonstrated a significant post-treatment effect in favour CFTR gene replacement therapy compared to placebo: at two months (gene therapy n = 60; placebo n = 52), MD 2.70% (95% CI 0.13 to 5.27); at three months (gene therapy n = 59; placebo n = 53), MD 3.96% (95% CI 1.34 to 6.58); at 11 months (gene therapy n = 58; placebo n = 52), MD 3.18% (95% CI 0.63 to 5.73); and at 12 months (gene therapy n = 60; placebo n = 54) MD 3.03% (95% CI 0.35 to 5.71) (Analysis 1.6). Data from the remaining monthly time points showed no significant difference between treatment groups (Alton 2015).

c. Relative change in FEV_1 or FVC

Only one study reported on relative change in FEV_1 % predicted as the primary endpoint, again at monthly intervals (Alton 2015). Results demonstrated small but significant benefits with gene replacement therapy: at two months (gene therapy n = 60; placebo n = 52), MD 3.05% (95% CI 0.14 to 5.96); at three months (gene therapy n = 59; placebo n = 53), MD 3.41% (95% CI 0.26 to 6.56); and at 12 months (gene therapy n = 60; placebo n = 54), MD 3.66% (95% CI 0.15 to 7.17) (Analysis 1.7). There was no significant difference between treatment groups at the remaining monthly time points (Analysis 1.7) (Alton 2015).

d. Other relevant lung function tests (e.g. infant lung function tests and lung clearance index)

One study reported on gas transfer (Alton 1999). This study reported a small but significant reduction in gas transfer in the CFTR gene replacement group, mean (SD) 7.5% (7.6), but not the placebo group, mean (SD) 1.6% (7.6) ($P < 0.05$) when measured on Day 2, which returned to baseline values when measured on Day 29, but did not present any actual data for this time point (Alton 1999).

Alton 2015 reported a non-significant treatment effect for diffusion capacity of the alveolar capillary membrane (KCOc), standardised treatment effect 0.23 (95% CI -0.17 to 0.63) (P = 0.257) and for transfer factor of the lung for carbon monoxide (TLCOc), standardised treatment effect 0.21 (95% CI -0.19 to 0.60) (P = 0.302) (both values corrected for haemoglobin concentrations) (Alton 2015). The same study also reported no significant difference in lung clearance index between treatment groups, standardised treatment effect 0.26 (95% CI -0.13 to 0.66) (P = 0.187); or on the mid-expiratory flow between 25% and 75% of FVC ($MEF_{25-75\%}$) for which the mean (SD) for the CFTR gene replacement group was -0.012 (0.395) and for the placebo group -0.08 (0.389), standardised treatment effect 0.18 (95% CI -0.21 to 0.56) (P = 0.362) (Alton 2015). They did not present values for each treatment group for these additional measures of lung function to include in the analysis (Alton 2015).

3. Survival

This was not reported as a specific outcome measure in any of the included studies. There were no deaths reported in either active or placebo groups in three studies (Alton 1999; Alton 2015; Moss 2004). In the remaining study, one participant in the placebo group died of an unrelated motorcycle accident (Moss 2007).

Secondary outcomes

1. Number of days as a hospital inpatient (or number of inpatient episodes)

No study reported the number of days as a hospital inpatient.
Moss 2004 did however report the number of inpatient episodes within 150 days (Moss 2004); there was no significant difference between groups (gene therapy n = 20; placebo n = 17) RR 0.85 (95% CI 0.37 to 1.94) (Analysis 1.8). It is not stated clearly in the study report whether these episodes are independent from each other. This outcome measure was not reported by the other included studies (Alton 1999; Alton 2015; Moss 2007).

2. Need for extra treatment

a. Physiotherapy (duration or episodes)

This outcome was not reported in any of the included studies.

3. Adverse events

Three studies reported adverse events as number of participants experiencing each adverse event, rather than absolute number of adverse event episodes (Alton 1999; Moss 2004; Moss 2007). The remaining study reported the mean number of times the respective adverse event was experienced by each participant (Alton 2015); it has therefore not been possible to combine results from this study with the earlier studies and insufficient data have been presented to report on Alton 2015 independently (Alton 2015). Due to the large number of different adverse events reported, we are only presenting significant results in the text below; for further details, please see the meta-analysis (Analysis 1.9).

a. Mild (e.g. sore throat, hoarse voice, dry mouth)

Neither Alton study showed a difference between the active and placebo groups in terms of mild adverse events. Alton 1999 presented the following events all occurring within 48 hours of dosing as mild: cough; wheeze; or tight chest and data for these events were pooled, making comparisons with other studies difficult (Alton 1999). Alton 2015 reported on headache and upper airway symptoms over the 12-month study period (Alton 2015). Both Moss studies individually recorded mild adverse events as rhinitis, pharyngitis, headache and sinusitis; again no significant differences between active and placebo groups were reported (Moss 2004; Moss 2007).

b. Moderate (e.g. wheeze, cough, fever, local allergic reaction)

Alton 1999 reported a significant increase in influenza-type symptoms in the CFTR gene replacement group resolving within 30 hours of dosing, RR 7.00 (95% CI 1.10 to 44.61) (Alton 1999). Alton 2015, one participant in each treatment group experienced "flu-like" symptoms; however this was defined as occurring following four or more treatment doses. It is not clear from the study why "flu-like" symptoms were only recorded after four or more treatment doses (Alton 2015). Alton 2015 assessed adverse events by the following predefined categories: lower airway symptoms, gastrointestinal symptoms, elevated liver function tests, haematuria and isolated raised inflammatory markers. They reported no significant difference between treatment groups for either total number of events or for specific adverse events by the aforementioned categories over 12 months. Data for the number of participants experiencing lower airway symptoms following four or more treatment doses were presented and have been included in analysis (Analysis 1.9); insufficient data were presented for the remaining categories for inclusion in our analyses (Alton 2015).
Moss 2004 assessed abdominal pain, asthma, chest pain, cough, increased cough, dyspnoea, fatigue, fever, decreased lung function and increased sputum, with no significant differences between active and placebo groups in any outcome to 150 days (Moss 2004).
Moss 2007 reported abdominal pain, asthma, increased cough, dyspnoea, fever, decreased lung function, and increased sputum; and also found no significant difference between groups to 210 days (Moss 2007).

c. Severe (e.g. coughing up blood (haemoptysis), collapsed lung (pneumothorax), chest infection, systemic allergic reactions)

Alton 1999 reported no severe adverse events in either the CFTR gene replacement or placebo groups (Alton 1999). There were no significant differences in incidence of severe adverse events between the groups in either Moss study (Moss 2004; Moss 2007). Alton 2015 reported six serious adverse events, all occurring in the CFTR gene therapy group. Only one event (admission to hospital with flu-like illness, pulmonary exacerbation and new isolate of MRSA within 24 hours of study bronchoscopy) was considered by their Data Monitoring and Ethics Committee and the Trial Steering Committee to be related to the study procedure (Alton 2015).

4. Quality of life

Only Alton 2015 reported on health-related quality of life (QoL) using the Cystic Fibrosis Questionnaire - Revised (CFQ-R) (Alton 2015). The study reported no significant post-treatment difference between treatment groups (CFTR gene therapy n = 61; placebo n = 54) for either physical functioning, MD 1.82 (95% CI -4.75 to 8.39) or respiratory symptoms, MD 2.08 (95% CI -3.06 to 7.22) (Alton 2015).

5. School or work attendance*

This outcome was not reported in any of the included studies.

6. Nutritional parameters

a. Weight

Only Alton 2015 reported on this outcome and found no clinically relevant changes in weight; however, it did not provide post-treatment data to analyse further (Alton 2015).

b. Body mass index (BMI)

This endpoint was only reported by Alton 2015, but no data were provided to allow analysis of this outcome; there were no clinically relevant changes in BMI (Alton 2015).

c. Height

This outcome was not reported in any of the included studies.

7. Acquisition of newly cultured respiratory pathogens

a. *Pseudomonas aeruginosa*

Two studies reported on this outcome (Alton 2015; Moss 2004). Alton 2015, 28 out of 60 (46.7%) participants in the CFTR gene therapy group were colonised with *Pseudomonas aeruginosa* (*P. aeruginosa*) at baseline compared to 27 out of 54 (50%) participants in the placebo group. No data were presented for the number of participants who were culture positive either at specific time points during the trial or at the end of the trial; but Alton reported that there were no clinically relevant changes in sputum microbiology, including *P. aeruginosa* (Alton 2015).

Moss 2004 reported on the culture of expectorated sputum at baseline and Day 90, but data are incomplete (Moss 2004). At baseline, 11 out of 16 (68.8%) participants in the gene therapy group were culture positive for *P. aeruginosa;* at Day 90, 14 participants from the gene therapy group were assessed and 10 of them were positive for *P. aeruginosa* (71.4%). In the placebo group, four of the eight participants assessed at baseline tested positive for *P. aeruginosa* (50%); at Day 90, eight of the 12 participants assessed tested positive for *P. aeruginosa* (66.7%). It is not possible to draw any conclusions from these limited data.

b. *Staphylococcus aureus*

Two studies reported on this outcome (Alton 2015; Moss 2004). Alton 2015 reported no clinically relevant changes in sputum microbiology of *Staphylococcus aureus;* no additional data were provided for analysis (Alton 2015).

Data collection in Moss 2004 was incomplete (Moss 2004). Of the six of the 16 participants in the gene therapy group assessed at baseline were culture positive for *Staphylococcus aureus* (37.5%). At Day 90, three of the 14 participants from the gene therapy group assessed were positive for *Staphylococcus aureus* (21.4%). In the placebo group, three out of eight participants assessed at baseline tested positive for *Staphylococcus aureus* (37.5%); at Day 90, seven of the 12 participants assessed tested positive for *Staphylococcus aureus* (58.3%). Again, it is not possible to draw any firm conclusions from these limited data.

c. *Haemophilus influenzae*

Only Alton 2015 reported on this outcome and found no clinically relevant changes in *Haemophilus influenzae* cultures (no further details were provided) (Alton 2015).

8. Sputum rheology

This outcome was not reported in any of the included studies.

9. Mucociliary clearance of the airway

Alton 2015 demonstrated this outcome by recording 24-hour sputum weight (Alton 2015). The study reported a non-significant difference in the change in 24-hour sputum weight between baseline and up to 12 months post treatment between the CFTR gene therapy group (n = 22) and the placebo group (n = 27), MD -2.96 (95% CI -7.97 to 2.05) (Alton 2015).

Alton 1999 reported simple saccharine nasal mucociliary clearance, but this is not an efficacy measure for CFTR gene replacement to the lung (Alton 1999).

10. Airway potential difference measurements (measuring salt transport)

Both Alton studies reported measurements of airway potential difference (Alton 1999; Alton 2015). These were measured in the lower airways using a bronchoscope and catheter. Both studies undertook bronchoscopy once before the administration of the study drug and repeated in Alton 1999 two days after the study drug was administered (Alton 1999) and at 28 days (plus or minus five days) after the final (12th) dose in Alton 2015 (Alton 2015). Alton 2015 also undertook an analysis of nasal potential difference in a subgroup of participants who were additionally treated with placebo or CFTR gene therapy via a nasal spray device; this has not been included in analysis as it is not an efficacy measure for CFTR gene replacement therapy to the lung (Alton 2015).

a. Baseline potential difference

People with CF have a higher baseline potential difference than people without CF (Griesenbach 2005). An effective CFTR gene replacement intervention would therefore be expected to demonstrate a reduction in baseline potential difference. Data for Alton 1999 are for segmental bronchi, estimated from figures in the original paper (Alton 1999). No significant change in baseline potential difference was seen in either the CFTR gene replacement group (n = 8), mean (SD) change -0.81 (9.02) millivolt (mV), or the placebo group (n = 8), mean (SD) change 2.53 (6.48) mV (Alton 1999). When these data are entered in the review's analysis, there was no difference between the groups, MD -3.34 (95% CI -11.04 to 4.36) (Analysis 1.10).

For Alton 2015, we calculated change in baseline potential difference from baseline and post-treatment data provided (mean (SEM)); assuming a correlation coefficient (Corr) of 0.9 to estimate the SD. There was no significant difference between treatment groups (CFTR gene therapy group n = 10; placebo n = 7), MD -0.30 (95% CI -2.90 to 2.30) (Analysis 1.10). Sensitivity analyses at a range of values for Corr also showed no statistical difference between treatment groups (Alton 2015). Alton 2015 demonstrated no significant difference in post-treatment (after 12 months) bronchial potential difference between treatment groups, MD 1.70 (95% CI -3.50 to 6.90) (Analysis 1.12). Data presented for this study are the mean of tracheal and all bronchial measurements (Alton 2015).

b. Response to perfusion with amiloride

Since sodium absorption is increased in people with CF, a greater reduction in potential difference from baseline is seen in response to amiloride in participants with CF than in those without CF (Griesenbach 2005). Thus effective CFTR gene replacement should reduce the fall in potential difference seen following amiloride. Alton 1999 demonstrated no significant change in response to perfusion with amiloride in either the CFTR gene replacement group (n = 8) mean (SD) change -12.6 (14.66) or the placebo group (n = 8) mean (SD) change -16.50 (13.17) (Alton 1999). Our analysis showed no difference between the groups, MD 3.90 (95% CI -9.76 to 17.56) (Analysis 1.11).

c. Response to perfusion with a zero chloride solution

The response to zero or low chloride secretion following prior perfusion with amiloride is the airway potential difference measurement that is the most effective discriminator between CF and non-CF participants (Alton 1999; Griesenbach 2005). Both Alton studies reported this as a summed response to low chloride solution and isoprenaline (Alton 1999; Alton 2015). A separate control group of participants without CF had a mean (SD) response of 10.70 (4.11). Data from Alton 1999 have been estimated from figures in the original paper (Alton 1999). The study reported that in the CFTR gene replacement group there was a significant improvement following perfusion with a zero chloride solution, mean (SD) 5.4 (3.82) ($P < 0.05$), with no significant change in the placebo group, mean (SD) -1.46 (2.29) (Alton 1999). This change in the CFTR replacement group (n = 8) was significantly different from that in the placebo group (n = 8) ($P < 0.0001$), MD 6.86 (95% CI 3.77 to 9.95) (Analysis 1.11).

In Alton 2015, participants in the placebo group (n=7) had a median (range) change of 3.1mV (9.3 to -1.2) compared to a median (range) change of -1.3mV (4.0 to -5.8) ($P = 0.032$) in the CFTR gene therapy group (n = 10). The mean change in bronchial chloride responses as well as pre- and post-treatment data for each individual participant were presented in graphical form; aggregate data were not available for analysis (Alton 2015).

11. Measures of gene expression

a. Quantitative reverse transcription polymerase chain reaction (rtPCR) to measure vector-specific CFTR messenger ribonucleic acid (mRNA) production

This outcome was reported in three studies, but at different time points (Alton 1999; Alton 2015; Moss 2004). In Alton 1999, bronchial brushings were performed two days following the single

dose of CFTR gene replacement or placebo. No vector specific mRNA could be detected by this measure in either the CFTR gene replacement group (n = 8), or the placebo group (n = 8) (Alton 1999). In Alton 2015, bronchial brushings were performed in a subgroup of participants at 28 days, plus or minus five days, after the 12th dose; four participants' measurements were taken beyond this time window due to clinical instability and one participant's bronchoscopy was performed after the sixth dose due to withdrawal from the study. No vector-specific mRNA was detected in either the CFTR gene replacement group (n = 14) or placebo group (n = 7) (Alton 2015). In Moss 2004, six out of the 20 participants in the active group and two out of the 17 participants in the placebo group had bronchial brushings performed 30 to 60 days after the third dose of the study drug. As with the previous two studies, no vector specific mRNA could be detected in any participant sampled (Moss 2004).

b. Any other method of measuring gene expression

Only Alton 2015 reported on this outcome and isolated DNA from bronchial brushings in the subgroup of participants who underwent bronchoscopy (Alton 2015). They demonstrated an increase in vector-specific DNA in 12 out of 14 participants (86%) in the CFTR gene replacement group compared to readings below the limit of quantification in the seven participants in the placebo group (P = 0.001) (Alton 2015).

12. Measures of CFTR protein expression

Only Alton 1999 measured chloride efflux in cells obtained by bronchial brushing, by measuring changes in 6-methoxy-N-(-sulphopropyl) quinolium (SPQ) fluorescence (Alton 1999). Any increase in CFTR protein expression following the delivery of the study drug would result in an increased efflux of the SPQ fluorescence. There was no significant difference in SPQ efflux between the two groups (CFTR gene therapy n = 6; placebo n = 7), MD 0.3 mMs^{-1} (95% CI -0.12 to 0.72) (Analysis 1.13). Data for the placebo group were estimated from a figure in the original paper. The original paper reports a significant increase in efflux (P < 0.05) in the active, but not in the placebo group (Alton 1999).

13. Radiological measures of lung disease

a. Validated chest radiograph scores

This outcome was only reported in Alton 1999, which used the previously validated CF Northern score (Conway 1994). The investigators report "no significant change from baseline" at Day 29, but the data are not shown (Alton 1999).

b. Validated computerised tomogram (CT) score

Two studies reported this outcome measure (Alton 2015; Moss 2004). In Alton 2015, high resolution CT scans were obtained from participants at baseline and at 28 days (plus or minus five days) after dose 12 of the study drug (Alton 2015). Investigators then studied the following features: extent of bronchiectasis (scored per lobe on a range of 0 to 3); severity of bronchiectasis and bronchial wall thickness (scored per lobe on a range of 0 to 4); small and large airway mucus plugs (scored per lobe on a range of 0 to 2); and gas trapping on expiratory CT (scored on a percentage basis). Results demonstrated a significant improvement in CT gas trapping for CFTR gene replacement therapy compared to placebo, MD -3.49 (95% CI -6.96 to -0.03) (Alton 2015). For the remaining features (extent of bronchiectasis, severity of bronchiectasis, bronchial wall thickening and small and large airway plugs), there was no significant difference in the change in score between treatment groups (Alton 2015).

In Moss 2004, participants underwent a high resolution CT scan at baseline and on Day 90, 30 days after the third dose of study drug had been administered (Moss 2004). The CT scans were scored on a scale from 0 to 100 using an algorithm that had not been previously validated and was developed for this study. There was no significant difference between the groups in terms of change in CT score from baseline at Day 90, with the CFTR gene replacement group (n = 18) mean (SD) -1.00 (3.00), placebo group (n = 17) mean (SD) 0.00 (6.00); MD -1.00 (95% CI -4.17 to 2.17) (Analysis 1.14).

These data must be treated with caution as the scoring system had not been previously validated.

DISCUSSION

Summary of main results

Four randomised controlled trials met the inclusion criteria for this review. They compared cystic fibrosis transmembrane conductance regulator (CFTR) gene replacement to placebo delivered to the lungs of participants with cystic fibrosis (CF) (Alton 1999; Alton 2015; Moss 2004; Moss 2007).

Two studies examined a liposome-based CFTR gene delivery agent (Alton 1999; Alton 2015). In Alton 1999 participants received a single dose of study drug with follow up for 29 days (Alton 1999) and in Alton 2015 participants received monthly doses for 12 months with follow up for up to 13 months (Alton 2015). In Alton 2015, at 12 months participants who received CFTR gene transfer agents had an improvement in relative change in forced expiratory volume at one second (FEV_1) % predicted compared to those who had received placebo, MD 3.66 (95% CI 0.15 to 7.17) (Analysis 1.7). This was also demonstrated after two and

three months, but not at the remaining time points over the 12-month study period (Alton 2015). Alton 2015 also demonstrated significant improvements in FVC (% predicted) at two, three, 11 and 12 months in participants receiving CFTR gene replacement therapy compared to placebo. Alton 1999 reported an significant increase in FVC (L) in the placebo group compared to the treatment group at up to 24 hours. With regards to adverse events, Alton 1999 reported "influenza-like" symptoms in seven out of eight participants who received the CFTR gene transfer reagents (Alton 1999). In the larger Alton 2015, these symptoms were reported in one participant who had taken at least four doses and one in the placebo arm (Alton 2015).

The two Moss studies used a viral CFTR gene delivery system (AAV) with follow up for 150 to 210 days respectively (Moss 2004; Moss 2007). These studies were of similar design, although three doses were given in the Moss 2004 and two doses in Moss 2007. There were no significant differences in rate of exacerbation between the treatment group and placebo in either study. Moss 2004 demonstrated a significant improvement in respiratory function (FEV_1) 30 days after participants had received their first dose of gene therapy agent. This finding was not confirmed in Moss 2007 or in our meta-analysis (Moss 2004; Moss 2007). Neither Moss study nor our meta-analysis of FVC (L) showed any significant difference between treatment and placebo groups at either 30 days or two months (Analysis 1.5).

No study reported on survival; however, no deaths were reported in three of the studies and the single death reported in one study was not treatment related.

Overall completeness and applicability of evidence

Four trials recruiting 302 participants with CF were included in this review; three trials recruited a mixture of males and females aged 12 years and over (Alton 2015; Moss 2004; Moss 2007) and one recruited only adult males due to regulatory restrictions (Alton 1999). The mean age of participants in the four studies was 24.5 years. Homozygous Δ F508 was the commonest genotype in the studies. There are insufficient data reported in the studies for some outcomes considered to be important for this review: oral antibiotics (days or episodes); number of days as a hospital inpatient; need for extra treatment (physiotherapy); school or work attendance; height; and sputum rheology.

Quality of the evidence

All included studies had a parallel design. Overall there were minor issues with quality, in particular risk of bias from concealment, allocation and randomisation. Methods of randomisation and allocation concealment are specified in just one study (Alton 2015); in the remaining studies, they are unclear (Alton 1999; Moss 2004; Moss 2007). In Moss 2004, 83% of randomised participants received all three doses of study drug and it is not stated whether withdrawals had been originally allocated to the active or the placebo group (Moss 2004). Post-allocation withdrawal also occurred in Moss 2007, but to a lesser extent study (Moss 2007). In Alton 2015, 83% of randomised participants received at least nine doses of study drug and were included in the per-protocol analysis. Reason for withdrawal was described up to the ninth dose, but it is not clear if participants withdrew after receiving nine doses (Alton 2015). In Alton 2015, measures were taken and described to ensure adequate blinding for the participants, but this was a challenge as the placebo was a quite distinct solution (saline) compared to the gene therapy agent (Alton 2015).

Potential biases in the review process

The review authors conducted a comprehensive literature search of online journal databases using the Cystic Fibrosis and Genetic Disorders Review Group's Cystic Fibrosis Trials Register and the ongoing online trials database (ClinicalTrials.gov). In addition, authors approached known researchers in the field for relevant unpublished information and individual participant data. Two authors (TWRL and KWS) selected eligible trials and extracted data for the original review and earlier updates. For the update in 2016, two authors (LAP and JCP-D) independently undertook further study selection and data extraction. The analyses of this update were undertaken by one review author (AA). This methodological approach ensured that risks of bias in the review process were kept to a minimum.

Agreements and disagreements with other studies or reviews

There are no other systematic reviews of topical gene replacement therapy for CF.

AUTHORS' CONCLUSIONS

Implications for practice

While the meta-analysis of most respiratory function tests showed no difference between treatment and placebo groups, the most recent study, Alton 2015, demonstrated a benefit in relative change in FEV_1 % predicted and FVC % predicted at 12 months for participants receiving CFTR gene replacement therapy compared to those receiving placebo (Alton 2015). Concerns over safety with "flu-like" symptoms in most participants in the Alton 1999 study were not reported on repeated use in Alton 2015 (Alton 1999; Alton 2015). There was no other evidence of positive impact on

outcomes, in particular improved quality of life, reduced treatment burden or exacerbations (Alton 2015). The limited evidence of efficacy does not support this as a routine therapy at present.

There was no evidence of efficacy for viral-mediated gene delivery from the remaining two included studies (Moss 2004; Moss 2007).

Implications for research

Clinical studies examining the use of CFTR gene transfer reagents for CF lung disease should include outcome measures that are relevant to people with CF as well as clearly defined surrogate measures of efficacy. It is imperative that future studies of established or novel gene therapy reagents use a robust study design with clearly defined endpoints and adequate power. These studies must be reported in a full and transparent manner to enable the incorporation of data into systematic reviews. A clear cost analysis is essential for any emerging therapies, as this is likely to be a significant factor. In future updates of this review, we will include this as a pertinent secondary outcome measure.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Alton 1999

Methods	Double-blind placebo-controlled RCT. Parallel design. Duration: 2 weeks. Single centre in UK.
Participants	16 participants (all male). Treatment group n = 8, placebo group n = 8 Mean age 26.9 years. Mean (SE) age: treatment group 27·5 (3·4) years, placebo group 26·3 (1·7) years. Confirmed CF, FEV_1 >70%, sterile. Genotype (not split by treatment/placebo group): Δ F508/Δ F508 n = 12 Δ F508/W1282X n = 1 Δ F508/other (i.e., no mutation detected after screening for mutations present in 92% to 94% of UK patients with CF) n = 3
Interventions	Single dose of CFTR DNA+liposome, or liposome alone nebulised to lungs. 1 week later administration to the nose
Outcomes	Adverse events, gene expression, CFTR protein expression, airway potential difference
Notes	Additional data requested from author, no additional data available. Analysed on intention-to-treat basis

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as randomised, but no further details given.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants and outcome assessors blinded.
Incomplete outcome data (attrition bias) All outcomes	High risk	All randomised participants assessed on intention-to-treat basis, although data incomplete for 4 reported endpoints
Selective reporting (reporting bias)	Unclear risk	Unclear.
Other bias	Unclear risk	Unclear.

Alton 2015

Methods	Double-blind, placebo-controlled RCT. Parallel design. Duration: 1 year. Multicentre (18 sites) in UK.
Participants	140 randomised. Confirmed CF aged 12 years and over and FEV_1 50% - 90% predicted. 2 participants from each group withdrew prior to treatment start so that: treatment group n = 76, placebo n = 60 Data available from 116 participants who received at least 9 doses: treatment group n = 62, placebo n = 54 Mean (SD) age: treatment group 23·6 (10·8) years; placebo group 26·0 (13·0) years Gender split (total: 56 males and 60 females): treatment group 31 males and 31 females; placebo 29 males and 25 females FEV_1 mean (SD): treatment group 69.9 (11.1); placebo group 69.0 (9.9) Height mean (SD): treatment group 163·6 (10·9) cm; placebo group 165·0 (10·6) cm Weight mean (SD): treatment group 61·0 (15·7) kg; placebo group 61·6 (15·6) kg BMI mean (SD): treatment group 22·4 (4·5) kg/m^2; placebo group 22·4 (4·4) kg/m^2. Mutation class: Phe508del/Phe508del: treatment group 31 (50%); placebo group 26 (48%). Phe508del/class 1-6: treatment group 23 (37%); placebo group 22 (41%). Not Phe508del/class 1: treatment group 3 (5%); placebo group 1 (2%). Heterozygous/homozygous class 3 - 6: treatment group 2 (3%); placebo group 2 (4%). Phe508del/unknown class: treatment group 3 (5%); placebo group 3 (6%)
Interventions	Treatment: 5 mL pGM169/GL67A (each dose contained 13·3 mg of plasmid DNA and 75 mg of the GL67A lipid mixture) Placebo: 5 mL placebo (0.9% saline). Treatment and placebo both nebulised to lungs through a Trudell AeroEclipse II device (Trudell Medical International, London, ON, Canada) 12 doses at 28 day intervals (plus or minus 5 days) for 12 months Routine treatments were continued throughout the trial, except for DNase, which was withheld for 24 hours before and after dosing
Outcomes	Primary outcome measure: relative change in % predicted FEV_1. Secondary outcome measures: additional measures of lung function; CT scan scores; CFQ-R scores; exercise testing; activity monitoring; sputum inflammatory markers; nasal or bronchial vector specific DNA or mRNA; electrophysiological assessment of CFTR function; adverse events
Notes	Sample size calculation undertaken. A total sample size of 120 assessable participants calculated to provide 90% power to detect a 6% difference between groups in the mean change from baseline at a two-sided 5% significance level Only the primary end point, relative change from baseline to end of treatment in FEV_1 % predicted, was reported as ITT.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

Random sequence generation (selection bias)	Low risk	Randomisation was performed, following a successful screening visit, via the on-line InForm database system (Oracle Health Sciences, Reading, UK). All data required for randomisation and stratification were entered by a member of the study team which led to the generation of a unique patient number, corresponding to a blinded, randomised arm of the study. In the event of computer system failure, a prearranged manual randomisation method was available from the InForm team
Allocation concealment (selection bias)	Low risk	Participants and investigators were masked to treatment allocation, with the randomisation code known only by pharmacy staff at the two dosing sites. The unique patient number was entered onto the participant's prescription sheet and submitted to the study pharmacists who had access to the unblinding code and prepared the active or placebo treatment as appropriate
Blinding (performance bias and detection bias) All outcomes	Unclear risk	5 ml volumes of pGM169 (13·25 mg)/GL67A (75 mg) or placebo (saline) were placed in AeroEclipse II breath-actuated nebulisers (Trudell Medical Instruments, London, ON, Canada). To avoid unblinding, nebulisers were taped and a tamper-proof seal was attached. Study medicines were prepared by unblinded trial pharmacists. Clinical study staff, participants and analysts were blind to allocation until database lock. 10 ml volumes were placed in opaque nasal spray devices (GSK parts No. AR5989 30 ml bottle/AR9488 30 ml actuator) and the device was primed. Details of consistency and taste of active trial drug versus placebo have not been provided
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	A total of 24 randomised participants withdrew from the study. Reasons given for all of these: 1. Discontinuation prior to initial dose (n = 4) Treatment group: 2 reconsidered participation/withdrew consent Placebo group: 1 reconsidered participation/withdrew consent and 1 was clinically

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		unstable 2. Discontinuation after initial dose after enrolment in ITT population (n = 20) Treatment group (n = 14): 5 due to time commitments, 1 wished to discontinue contraception, 1 changed mind regarding participation, 1 had borderline FEV_1 at screening and throughout study, 1 lived in nomadic community and not available for follow up, 1 had a new culture of MRSA, 1 had a new culture of *Mycobacterium abscessus*, 1 had a new culture of *Burkholderia cepacia* and 2 commenced ivacaftor. Placebo group (n = 6): 2 due to time commitments, 1 disliked venepuncture, 1 had increased respiratory adverse events, 1 had a new culture of *Mycobacterium abscessus* and 1 commenced ivacaftor.
Selective reporting (reporting bias)	Low risk	Renal function and anti-nuclear antibodies (as a marker of immune response) were stated in the protocol but not reported in the paper
Other bias	Low risk	Similar baseline characteristics.

Moss 2004

Methods	Double-blind placebo-controlled RCT. Parallel design. Duration: 3 times (30 day interval). Multicentre (8 CF centres) in the USA.
Participants	37 participants (15 male, 22 female). Treatment group n = 20 (6 male, 14 female), placebo group n = 17 (9 male, 8 female). Mean age 23.7 years. Mean (SD) age: in treatment group 24.2 (8.7) years; in placebo group 23.2(11.1) years. Confirmed CF, FEV_1 > 60%. Mean (SD) FEV_1: in treatment group 82.2 (19.3) and in placebo group 84.4 (15.1),P = 0.70 Genotype: F508 homozygous: treatment group 5 (25%), placebo group 13 (77%) F508 heterozygous: treatment group 12 (60%), placebo group 2 (12%) Other/unknown: treatment group 3 (15%), placebo group 2 (12%)
Interventions	Treatment: 1x10(13) particles tgAAVCF 3 times (30 day interval) nebulised to lungs Control: matching placebo 3 times (30 day interval) nebulised to lungs
Outcomes	Respiratory exacerbations, adverse events, lung function, inpatient episodes, acquisition of new pathogens, gene expression, change in CT score

Notes	Analysed on intention-to-treat basis. Additional data requested from author, original data provided Power calculation undertaken - paper states "Enrollment of 18 subjects per treatment group provided adequate power to test differences between treatment groups with respect to the primary and secondary protocol end points"

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as randomised, but no further details given.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blind, no further details.
Incomplete outcome data (attrition bias) All outcomes	High risk	Analysed on intention-to-treat basis. 42 participants randomised, 37 received at least one dose of study drug, 35 completed all 3 doses. Not clear if withdrawals in placebo or active group, data pooled for 37 participants receiving at least 1 dose. Only 35 participants underwent HRCT lung scans, and just 10 had vector-specific CFTR gene expression assessed The first participant, who had received 1 dose of study medication, was withdrawn from the study as a result of a FDA hold on the research. The second participant was withdrawn prior to dosing, then later was re-screened and was re-randomized, but withdrew consent prior to dosing. 3 other participants withdrew consent after randomization but prior to starting treatment because of heavy work schedule, concern about potential risks, and a change of mind, respectively
Selective reporting (reporting bias)	Unclear risk	Unclear.
Other bias	High risk	The placebo group had significantly more ΔF508 homozygous participants (77%) than the CFTR gene replacement group (25%) (P = 0.01)

Moss 2007

Methods	Double-blind placebo-controlled RCT. Parallel design. Duration: 2 times (30-day interval). Multicentre (12 centres) in USA.
Participants	122 people screened, 109 randomised and 102 participants (54 male, 48 female) received treatment. 98 completed study and 4 stopped early (reasons given - see below) Treatment group n = 51 (26 male), placebo group n = 51 (28 male). Mean age 22.6 years, all aged over 12 years. Mean (SD) age: treatment group 23.9 (10.9) years, placebo group 21.3 (8.7) years. Confirmed CF, FEV_1 > 60% predicted. Mean (SD) FEV_1 % predicted: treatment group 84.7 (13.7), placebo group 87.9 (15.5) Genotype: AF508 homozygous: treatment group 27 (53%), placebo group 27 (53%) AF508 heterozygous: treatment group 18 (35%), placebo group 20 (39%)
Interventions	Treatment: 1x10(13) particles tgAAVCF 2 times (30 day interval) nebulised to lungs Control: matching placebo 2 times (30 day interval) nebulised to lungs
Outcomes	Respiratory exacerbations, adverse events, lung function.
Notes	Analysed on intention-to-treat basis. Additional data requested from author, original data provided Sample size calculation based on the initial phase 2 multidose aerosol study. "Enrollment of 100 subjects, 50 in each treatment arm, would provide 93% power to detect a 0.14 L difference in the 30-day change in FEV_1 between treatment groups, assuming a standard deviation of the change of 0.20 L in each group. This sample size would also provide adequate power for detecting 0.3 log10-ng/ml differences between treatment groups in IL-8."

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as randomised, but no further details given.
Allocation concealment (selection bias)	Unclear risk	Not discussed.
Blinding (performance bias and detection bias) All outcomes	Low risk	Double-blind, no further details.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Intention-to-treat basis. 109 participants randomised, of whom 102 received at least 1 dose of study drug, 98 completed trial - 4 participants stopped early. Of these, 1 was in the tgAAVCF treatment group and 3 were in the placebo group. The reason the

		participant from the tgAAVCF group discontinued was loss to follow-up. Reasons for discontinuation in the 3 placebo recipients included experiencing an adverse event (unlikely to be related pulmonary exacerbation), death (unrelated motorcycle accident), and other (no response to day 210 phone call)
Selective reporting (reporting bias)	High risk	Some relevant secondary endpoints, such as number of days of oral antibiotic use, remain unreported
Other bias	Unclear risk	Unclear.

CF: cystic fibrosis
CFTR: cystic fibrosis transmembrane conductance regulator
CT: computerised tomogram scan (of chest)
DNA: deoxyribonucleic acid
DNase: dornase alfa
FEV_1: forced expiratory volume in one second
ITT: intention-to-treat
MRSA: methicillin-resistant *Staphylococcus aureus*
RCT: randomised controlled trial
SD: standard deviation
tgAAVCF: Targeted Genetics Corporation adeno-associated virus encoding CFTR

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Davies 2011	Not a RCT.
Flotte 1996	Not a RCT.
Gill 1997	Applied to nose not to lungs.
Harvey 1999	Not a RCT.
Hyde 2000	Applied to nose not to lungs.
Joseph 2001	Not a RCT.
Knowles 1995	Applied to nose not to lungs.

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Noone 2000	Not a RCT.
Porteous 1997	Applied to nose not to lungs.
Wagner 1999	Applied to sinuses not lungs.
Wagner 2002	Applied to sinuses not lungs.
Zabner 1996	Not a RCT.
Zabner 1997	Not a RCT.
Zuckerman 1999	Not a RCT.

RCT: randomised controlled trial

DATA AND ANALYSES

Comparison 1. CFTR gene replacement therapy versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Respiratory exacerbations (episodes)	2		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
1.1 5 - 6 months	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
1.2 6 - 9 months	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2 Respiratory exacerbations (number of participants)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
2.1 9 - 12 months	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3 Change in FEV1 (L) from baseline	3		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
3.1 At <24 hours	1	16	Mean Difference (IV, Fixed, 95% CI)	-1.40 [-3.07, 0.27]
3.2 At up to 30 days	2	139	Mean Difference (IV, Fixed, 95% CI)	0.06 [-0.00, 0.13]
3.3 At up to 2 months	2	138	Mean Difference (IV, Fixed, 95% CI)	-0.05 [-0.12, 0.02]
4 Change in FEV1 % predicted from baseline	2		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
4.1 At up to 30 days	2	139	Mean Difference (IV, Fixed, 95% CI)	1.48 [-0.43, 3.39]
4.2 At up to 2 months	2	138	Mean Difference (IV, Fixed, 95% CI)	-2.03 [-4.21, 0.14]
4.3 At up to 3 months	1	37	Mean Difference (IV, Fixed, 95% CI)	1.25 [-3.84, 6.34]
5 Change in FVC (L)	3		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
5.1 At <24 hours	1	16	Mean Difference (IV, Fixed, 95% CI)	-1.70 [-3.27, -0.13]
5.2 At up to 30 days	2	139	Mean Difference (IV, Fixed, 95% CI)	0.02 [-0.05, 0.09]
5.3 At up to 2 months	2	138	Mean Difference (IV, Fixed, 95% CI)	-0.06 [-0.13, 0.02]
6 Change in FVC (% predicted) from baseline	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
6.1 At up to 30 days	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.2 At up to 2 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.3 At up to 3 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.4 At up to 4 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.5 At up to 5 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.6 At up to 6 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.7 At up to 7 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.8 At up to 8 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.9 At up to 9 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.10 At up to 10 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.11 At up to 11 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.12 At up to 12 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7 Relative change in FEV_1 % predicted from baseline	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
7.1 At up to 30 days	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.2 At up to 2 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.3 At up to 3 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.4 At up to 4 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.5 At up to 5 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.6 At up to 6 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]

7.7 At up to 7 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.8 At up to 8 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.9 At up to 9 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.10 At up to 10 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.11 At up to 11 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.12 At up to 12 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
8 Number of inpatient episodes	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
8.1 Within 5 - 6 months	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
9 Adverse events	4		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
9.1 Mild airway symptoms (Alton-pooled: Cough, wheeze, tight chest)	1	16	Risk Ratio (M-H, Fixed, 95% CI)	1.0 [0.57, 1.76]
9.2 Rhinitis	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.67, 1.14]
9.3 Pharyngitis	2	139	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.76, 1.70]
9.4 Headache	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.51, 1.45]
9.5 Sinusitis	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.53, 1.54]
9.6 Moderate: Influenza type symptoms (Alton: pooled)	2	132	Risk Ratio (M-H, Fixed, 95% CI)	3.83 [0.96, 15.33]
9.7 Abdominal pain	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.36, 1.20]
9.8 Asthma	2	139	Risk Ratio (M-H, Fixed, 95% CI)	1.94 [0.48, 7.91]
9.9 Chest pain	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.50, 1.57]
9.10 Cough	1	37	Risk Ratio (M-H, Fixed, 95% CI)	6.0 [0.33, 108.56]
9.11 Increased cough	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.71, 1.10]
9.12 Dyspnoea	2	139	Risk Ratio (M-H, Fixed, 95% CI)	1.03 [0.38, 2.84]
9.13 Fatigue	1	37	Risk Ratio (M-H, Fixed, 95% CI)	0.97 [0.44, 2.12]
9.14 Fever	2	139	Risk Ratio (M-H, Fixed, 95% CI)	1.36 [0.79, 2.36]
9.15 Decreased lung function	2	139	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.63, 2.18]
9.16 Increased Sputum	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.61, 1.20]
9.17 Severe (Alton: pooled)	1	16	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
9.18 CF lung exacerbation	2	139	Risk Ratio (M-H, Fixed, 95% CI)	1.40 [0.75, 2.61]
9.19 Hemoptysis	2	139	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.32, 2.47]
9.20 Lung disorder	1	37	Risk Ratio (M-H, Fixed, 95% CI)	1.28 [0.43, 3.78]
9.21 Lower airway symptoms	1	116	Risk Ratio (M-H, Fixed, 95% CI)	1.99 [0.89, 4.47]
10 Lower airway potential difference change from baseline	2		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
10.1 At up to 30 days	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
10.2 At up to 13 months Corr 0.1	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
10.3 At up to 13 months Corr 0.5	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
10.4 At up to 13 months Corr 0.9	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
11 Lower airway potential difference change from baseline (amiloride and low chloride)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
11.1 Response to perfusion with amiloride	1	16	Mean Difference (IV, Fixed, 95% CI)	3.90 [-9.76, 17.56]
11.2 Response to perfusion with zero chloride solution	1	16	Mean Difference (IV, Fixed, 95% CI)	6.86 [3.77, 9.95]
12 Lower airway potential difference - post treatment	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
12.1 At up to 12 months	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]

13 Measurement of CFTR protein expression (SPQ chloride efflux)	1	Mean Difference (IV, Fixed, 95% CI)	Subtotals only
14 Change in validated computerised tomogram (CT) score	1	Mean Difference (IV, Fixed, 95% CI)	Subtotals only

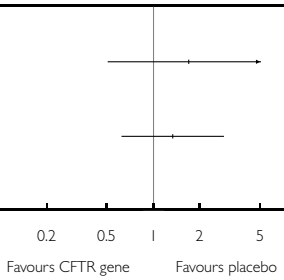
Analysis 1.1. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 1 Respiratory exacerbations (episodes).

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 1 Respiratory exacerbations (episodes)

Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 5 - 6 months				
Moss 2004	6/20	3/17		1.70 [0.50, 5.79]
2 6 - 9 months				
Moss 2007	12/51	9/51		1.33 [0.62, 2.89]



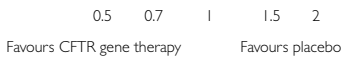
Analysis 1.2. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 2 Respiratory exacerbations (number of participants).

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 2 Respiratory exacerbations (number of participants)

Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 9 - 12 months				
Alton 2015	30/59	26/54		1.06 [0.73, 1.53]



Analysis 1.3. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 3 Change in FEV1 (L) from baseline.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 3 Change in FEV1 (L) from baseline

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
1 At <24 hours							
Alton 1999	8	-16.7 (1.7)	8	-15.3 (1.7)		100.0 %	-1.40 [-3.07, 0.27]
Subtotal (95% CI)	**8**		**8**			**100.0 %**	**-1.40 [-3.07, 0.27]**
Heterogeneity: not applicable							
Test for overall effect: Z = 1.65 (P = 0.10)							
2 At up to 30 days							
Moss 2004	20	0.1 (0.2)	17	-0.04 (0.18)		30.0 %	0.14 [0.02, 0.26]
Moss 2007	51	0 (0.23)	51	-0.03 (0.18)		70.0 %	0.03 [-0.05, 0.11]
Subtotal (95% CI)	**71**		**68**			**100.0 %**	**0.06 [0.00, 0.13]**
Heterogeneity: Chi^2 = 2.17, df = 1 (P = 0.14); I^2 =54%							

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Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Test for overall effect: Z = 1.84 (P = 0.066)							
3 At up to 2 months							
Moss 2004	19	0.02 (0.27)	17	-0.03 (0.24)		17.9 %	0.05 [-0.12, 0.22]
Moss 2007	51	-0.03 (0.21)	51	0.04 (0.19)		82.1 %	-0.07 [-0.15, 0.01]
Subtotal (95% CI)	**70**		**68**			**100.0 %**	**-0.05 [-0.12, 0.02]**
Heterogeneity: Chi^2 = 1.64, df = 1 (P = 0.20); I^2 =39%							
Test for overall effect: Z = 1.35 (P = 0.18)							
Test for subgroup differences: Chi^2 = 7.80, df = 2 (P = 0.02), I^2 =74%							

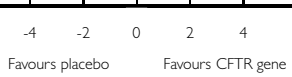
Analysis 1.4. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 4 Change in FEV1 % predicted from baseline.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 4 Change in FEV1 % predicted from baseline

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
1 At up to 30 days							
Moss 2004	20	2.26 (5.92)	17	-0.73 (4.73)		31.0 %	2.99 [-0.44, 6.42]
Moss 2007	51	-0.42 (6.37)	51	-1.22 (5.46)		69.0 %	0.80 [-1.50, 3.10]
Subtotal (95% CI)	**71**		**68**			**100.0 %**	**1.48 [-0.43, 3.39]**
Heterogeneity: Chi^2 = 1.08, df = 1 (P = 0.30); I^2 =7%							
Test for overall effect: Z = 1.52 (P = 0.13)							
2 At up to 2 months							
Moss 2004	19	0.07 (7.48)	17	-0.32 (6.92)		21.3 %	0.39 [-4.31, 5.09]
Moss 2007	51	-1.82 (7.11)	51	0.87 (5.39)		78.7 %	-2.69 [-5.14, -0.24]



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Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Subtotal (95% CI)	**70**		**68**			**100.0 %**	**-2.03 [-4.21, 0.14]**
Heterogeneity: Chi^2 = 1.30, df = 1 (P = 0.26); I^2 =23%							
Test for overall effect: Z = 1.83 (P = 0.067)							
3 At up to 3 months							
Moss 2004	20	-1.28 (8.3)	17	-2.53 (7.5)		100.0 %	1.25 [-3.84, 6.34]
Subtotal (95% CI)	**20**		**17**			**100.0 %**	**1.25 [-3.84, 6.34]**
Heterogeneity: not applicable							
Test for overall effect: Z = 0.48 (P = 0.63)							
Test for subgroup differences: Chi^2 = 5.89, df = 2 (P = 0.05), I^2 =66%							

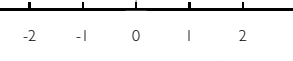
Analysis 1.5. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 5 Change in FVC (L).

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 5 Change in FVC (L)

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
1 At <24 hours							
Alton 1999	8	-16.6 (1.98)	8	-14.9 (1.1)		100.0 %	-1.70 [-3.27, -0.13]
Subtotal (95% CI)	**8**		**8**			**100.0 %**	**-1.70 [-3.27, -0.13]**
Heterogeneity: not applicable							
Test for overall effect: Z = 2.12 (P = 0.034)							
2 At up to 30 days							
Moss 2004	20	0.06 (0.2)	17	-0.07 (0.25)		23.4 %	0.13 [-0.02, 0.28]
Moss 2007	51	0 (0.2)	51	0.01 (0.22)		76.6 %	-0.01 [-0.09, 0.07]
Subtotal (95% CI)	**71**		**68**			**100.0 %**	**0.02 [-0.05, 0.09]**
Heterogeneity: Chi^2 = 2.65, df = 1 (P = 0.10); I^2 =62%							



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Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Test for overall effect: Z = 0.62 (P = 0.53)							
3 At up to 2 months							
Moss 2004	19	0.01 (0.28)	17	-0.01 (0.28)		15.3 %	0.02 [-0.16, 0.20]
Moss 2007	51	-0.01 (0.19)	51	0.06 (0.21)		84.7 %	-0.07 [-0.15, 0.01]
Subtotal (95% CI)	**70**		**68**			**100.0 %**	**-0.06 [-0.13, 0.02]**
Heterogeneity: Chi^2 = 0.79, df = 1 (P = 0.38); I^2 =0.0%							
Test for overall effect: Z = 1.54 (P = 0.12)							
Test for subgroup differences: Chi^2 = 6.76, df = 2 (P = 0.03), I^2 =70%							

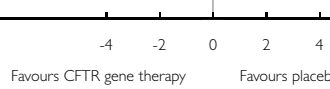
Analysis 1.6. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 6 Change in FVC (% predicted) from baseline.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 6 Change in FVC (% predicted) from baseline

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At up to 30 days						
Alton 2015	62	0.82 (6.86)	52	-0.74 (6.87)		1.56 [-0.97, 4.09]
2 At up to 2 months						
Alton 2015	60	0.79 (6.92)	52	-1.91 (6.93)		2.70 [0.13, 5.27]
3 At up to 3 months						
Alton 2015	59	1.01 (7.07)	53	-2.95 (7.08)		3.96 [1.34, 6.58]
4 At up to 4 months						
Alton 2015	61	0.37 (7.26)	51	-1.09 (7.28)		1.46 [-1.24, 4.16]
5 At up to 5 months						



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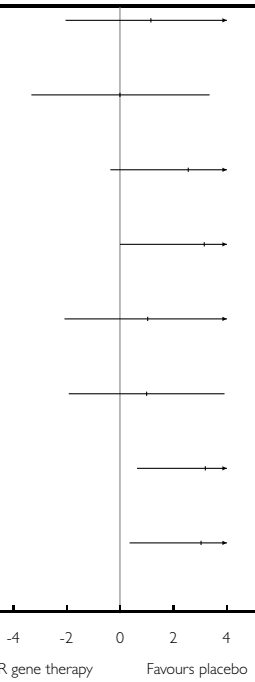
Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
Alton 2015	56	0.84 (8.42)	51	-0.31 (8.43)		1.15 [-2.05, 4.35]
6 At up to 6 months						
Alton 2015	57	-0.73 (8.71)	49	-0.74 (8.73)		0.01 [-3.32, 3.34]
7 At up to 7 months						
Alton 2015	55	1.52 (7.78)	53	-1.04 (7.78)		2.56 [-0.38, 5.50]
8 At up to 8 months						
Alton 2015	55	1.99 (8.17)	48	-1.17 (8.18)		3.16 [0.00, 6.32]
9 At up to 9 months						
Alton 2015	59	0.41 (8.26)	51	-0.61 (8.28)		1.02 [-2.08, 4.12]
10 At up to 10 months						
Alton 2015	59	0.7 (7.7)	49	-0.28 (7.71)		0.98 [-1.94, 3.90]
11 At up to 11 months						
Alton 2015	58	0.86 (6.82)	52	-2.32 (6.83)		3.18 [0.63, 5.73]
12 At up to 12 months						
Alton 2015	60	0.5 (7.3)	54	-2.53 (7.3)		3.03 [0.35, 5.71]



Analysis 1.7. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 7 Relative change in FEV1 % predicted from baseline.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 7 Relative change in FEV_1 % predicted from baseline

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At up to 30 days						
Alton 2015	62	0.92 (8.73)	53	-1.66 (8.74)		2.58 [-0.62, 5.78]
2 At up to 2 months						
Alton 2015	60	0.42 (7.83)	52	-2.63 (7.85)		3.05 [0.14, 5.96]
3 At up to 3 months						
Alton 2015	59	0.61 (8.5)	53	-2.8 (8.51)		3.41 [0.26, 6.56]
4 At up to 4 months						
Alton 2015	61	-0.39 (8.69)	51	-1.45 (8.72)		1.06 [-2.18, 4.30]
5 At up to 5 months						
Alton 2015	56	0.38 (8.59)	51	0.05 (8.6)		0.33 [-2.93, 3.59]
6 At up to 6 months						
Alton 2015	58	-0.57 (10.81)	49	-1.42 (10.82)		0.85 [-3.26, 4.96]
7 At up to 7 months						
Alton 2015	56	1.56 (10.26)	53	-2.14 (10.26)		3.70 [-0.15, 7.55]
8 At up to 8 months						
Alton 2015	55	1.09 (10.14)	48	-1.16 (10.15)		2.25 [-1.68, 6.18]
9 At up to 9 months						
Alton 2015	59	-1.54 (9.69)	51	-1.63 (9.71)		0.09 [-3.55, 3.73]
10 At up to 10 months						
Alton 2015	59	-0.29 (11.39)	49	-1.65 (11.42)		1.36 [-2.96, 5.68]
11 At up to 11 months						
Alton 2015	58	-0.02 (9.32)	52	-3.32 (9.33)		3.30 [-0.19, 6.79]
12 At up to 12 months						
Alton 2015	60	-0.35 (9.54)	54	-4.01 (9.55)		3.66 [0.15, 7.17]

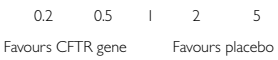
Analysis 1.8. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 8 Number of inpatient episodes.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 8 Number of inpatient episodes

Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Within 5 - 6 months				
Moss 2004	7/20	7/17		0.85 [0.37, 1.94]



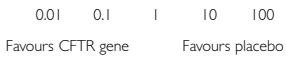
Analysis 1.9. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 9 Adverse events.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 9 Adverse events

Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Mild airway symptoms (Alton-pooled: Cough, wheeze, tight chest)					
Alton 1999	6/8	6/8		100.0 %	1.00 [0.57, 1.76]
Subtotal (95% CI)	**8**	**8**		**100.0 %**	**1.00 [0.57, 1.76]**
Total events: 6 (CFTR gene therapy), 6 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.0 (P = 1.0)					
2 Rhinitis					
Moss 2004	11/20	10/17		23.6 %	0.94 [0.53, 1.64]
Moss 2007	30/51	35/51		76.4 %	0.86 [0.64, 1.15]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.88 [0.67, 1.14]**
Total events: 41 (CFTR gene therapy), 45 (Placebo)					
Heterogeneity: Chi^2 = 0.07, df = 1 (P = 0.79); I^2 =0.0%					



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Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 1.00 (P = 0.32)					
3 Pharyngitis					
Moss 2004	9/20	7/17		28.5 %	1.09 [0.52, 2.31]
Moss 2007	22/51	19/51		71.5 %	1.16 [0.72, 1.86]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**1.14 [0.76, 1.70]**
Total events: 31 (CFTR gene therapy), 26 (Placebo)					
Heterogeneity: Chi^2 = 0.02, df = 1 (P = 0.90); I^2 =0.0%					
Test for overall effect: Z = 0.64 (P = 0.52)					
4 Headache					
Moss 2004	7/20	6/17		30.2 %	0.99 [0.41, 2.39]
Moss 2007	12/51	15/51		69.8 %	0.80 [0.42, 1.54]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.86 [0.51, 1.45]**
Total events: 19 (CFTR gene therapy), 21 (Placebo)					
Heterogeneity: Chi^2 = 0.15, df = 1 (P = 0.70); I^2 =0.0%					
Test for overall effect: Z = 0.57 (P = 0.57)					
5 Sinusitis					
Moss 2004	7/20	5/17		26.5 %	1.19 [0.46, 3.07]
Moss 2007	12/51	15/51		73.5 %	0.80 [0.42, 1.54]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.90 [0.53, 1.54]**
Total events: 19 (CFTR gene therapy), 20 (Placebo)					
Heterogeneity: Chi^2 = 0.46, df = 1 (P = 0.50); I^2 =0.0%					
Test for overall effect: Z = 0.37 (P = 0.71)					
6 Moderate: Influenza type symptoms (Alton: pooled)					
Alton 1999	7/8	1/8		48.3 %	7.00 [1.10, 44.61]
Alton 2015	1/62	1/54		51.7 %	0.87 [0.06, 13.59]
Subtotal (95% CI)	**70**	**62**		**100.0 %**	**3.83 [0.96, 15.33]**
Total events: 8 (CFTR gene therapy), 2 (Placebo)					
Heterogeneity: Chi^2 = 1.52, df = 1 (P = 0.22); I^2 =34%					
Test for overall effect: Z = 1.90 (P = 0.057)					
7 Abdominal pain					
Moss 2004	6/20	6/17		31.7 %	0.85 [0.34, 2.15]
Moss 2007	8/51	14/51		68.3 %	0.57 [0.26, 1.24]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.66 [0.36, 1.20]**
Total events: 14 (CFTR gene therapy), 20 (Placebo)					
Heterogeneity: Chi^2 = 0.42, df = 1 (P = 0.52); I^2 =0.0%					
Test for overall effect: Z = 1.37 (P = 0.17)					
8 Asthma					
Moss 2004	4/20	2/17		81.2 %	1.70 [0.35, 8.17]
Moss 2007	1/51	0/51		18.8 %	3.00 [0.13, 71.96]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**1.94 [0.48, 7.91]**

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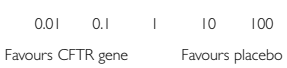
Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Total events: 5 (CFTR gene therapy), 2 (Placebo)					
Heterogeneity: Chi² = 0.10, df = 1 (P = 0.75); I² =0.0%					
Test for overall effect: Z = 0.93 (P = 0.35)					
9 Chest pain					
Moss 2004	6/20	7/17		40.8 %	0.73 [0.30, 1.75]
Moss 2007	11/51	11/51		59.2 %	1.00 [0.48, 2.10]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.89 [0.50, 1.57]**
Total events: 17 (CFTR gene therapy), 18 (Placebo)					
Heterogeneity: Chi² = 0.29, df = 1 (P = 0.59); I² =0.0%					
Test for overall effect: Z = 0.41 (P = 0.69)					
10 Cough					
Moss 2004	3/20	0/17		100.0 %	6.00 [0.33, 108.56]
Subtotal (95% CI)	**20**	**17**		**100.0 %**	**6.00 [0.33, 108.56]**
Total events: 3 (CFTR gene therapy), 0 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.21 (P = 0.23)					
11 Increased cough					
Moss 2004	12/20	12/17		25.5 %	0.85 [0.53, 1.36]
Moss 2007	34/51	38/51		74.5 %	0.89 [0.70, 1.15]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.88 [0.71, 1.10]**
Total events: 46 (CFTR gene therapy), 50 (Placebo)					
Heterogeneity: Chi² = 0.04, df = 1 (P = 0.85); I² =0.0%					
Test for overall effect: Z = 1.09 (P = 0.27)					
12 Dyspnoea					
Moss 2004	6/20	4/17		74.2 %	1.28 [0.43, 3.78]
Moss 2007	0/51	1/51		25.8 %	0.33 [0.01, 8.00]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**1.03 [0.38, 2.84]**
Total events: 6 (CFTR gene therapy), 5 (Placebo)					
Heterogeneity: Chi² = 0.63, df = 1 (P = 0.43); I² =0.0%					
Test for overall effect: Z = 0.06 (P = 0.95)					
13 Fatigue					
Moss 2004	8/20	7/17		100.0 %	0.97 [0.44, 2.12]
Subtotal (95% CI)	**20**	**17**		**100.0 %**	**0.97 [0.44, 2.12]**
Total events: 8 (CFTR gene therapy), 7 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.07 (P = 0.94)					
14 Fever					
Moss 2004	8/20	8/17		55.3 %	0.85 [0.41, 1.78]
Moss 2007	14/51	7/51		44.7 %	2.00 [0.88, 4.54]



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Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**1.36 [0.79, 2.36]**
Total events: 22 (CFTR gene therapy), 15 (Placebo)					
Heterogeneity: Chi² = 2.42, df = 1 (P = 0.12); I² =59%					
Test for overall effect: Z = 1.11 (P = 0.27)					
15 Decreased lung function					
Moss 2004	3/20	4/17		30.2 %	0.64 [0.17, 2.46]
Moss 2007	14/51	10/51		69.8 %	1.40 [0.69, 2.86]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**1.17 [0.63, 2.18]**
Total events: 17 (CFTR gene therapy), 14 (Placebo)					
Heterogeneity: Chi² = 1.02, df = 1 (P = 0.31); I² =2%					
Test for overall effect: Z = 0.49 (P = 0.62)					
16 Increased Sputum					
Moss 2004	13/20	12/17		36.1 %	0.92 [0.59, 1.44]
Moss 2007	19/51	23/51		63.9 %	0.83 [0.52, 1.32]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.86 [0.61, 1.20]**
Total events: 32 (CFTR gene therapy), 35 (Placebo)					
Heterogeneity: Chi² = 0.12, df = 1 (P = 0.73); I² =0.0%					
Test for overall effect: Z = 0.88 (P = 0.38)					
17 Severe (Alton: pooled)					
Alton 1999	0/8	0/8			Not estimable
Subtotal (95% CI)	**8**	**8**			**Not estimable**
Total events: 0 (CFTR gene therapy), 0 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
18 CF lung exacerbation					
Moss 2004	6/20	3/17		24.5 %	1.70 [0.50, 5.79]
Moss 2007	13/51	10/51		75.5 %	1.30 [0.63, 2.69]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**1.40 [0.75, 2.61]**
Total events: 19 (CFTR gene therapy), 13 (Placebo)					
Heterogeneity: Chi² = 0.14, df = 1 (P = 0.71); I² =0.0%					
Test for overall effect: Z = 1.05 (P = 0.29)					
19 Hemoptysis					
Moss 2004	5/20	4/17		68.4 %	1.06 [0.34, 3.34]
Moss 2007	1/51	2/51		31.6 %	0.50 [0.05, 5.34]
Subtotal (95% CI)	**71**	**68**		**100.0 %**	**0.88 [0.32, 2.47]**
Total events: 6 (CFTR gene therapy), 6 (Placebo)					
Heterogeneity: Chi² = 0.32, df = 1 (P = 0.57); I² =0.0%					
Test for overall effect: Z = 0.23 (P = 0.81)					

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Study or subgroup	CFTR gene therapy n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
20 Lung disorder					
Moss 2004	6/20	4/17		100.0 %	1.28 [0.43, 3.78]
Subtotal (95% CI)	**20**	**17**		**100.0 %**	**1.28 [0.43, 3.78]**
Total events: 6 (CFTR gene therapy), 4 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.44 (P = 0.66)					
21 Lower airway symptoms					
Alton 2015	16/62	7/54		100.0 %	1.99 [0.89, 4.47]
Subtotal (95% CI)	**62**	**54**		**100.0 %**	**1.99 [0.89, 4.47]**
Total events: 16 (CFTR gene therapy), 7 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.67 (P = 0.096)					

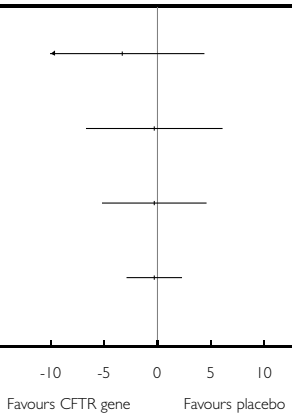
Analysis 1.10. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 10 Lower airway potential difference change from baseline.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 10 Lower airway potential difference change from baseline

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At up to 30 days						
Alton 1999	8	-0.81 (9.02)	8	2.53 (6.48)		-3.34 [-11.04, 4.36]
2 At up to 13 months Corr 0.1						
Alton 2015	10	1.8 (7.21)	7	2.1 (6.24)		-0.30 [-6.73, 6.13]
3 At up to 13 months Corr 0.5						
Alton 2015	10	1.8 (5.38)	7	2.1 (4.86)		-0.30 [-5.21, 4.61]
4 At up to 13 months Corr 0.9						
Alton 2015	10	1.8 (2.4)	7	2.1 (2.88)		-0.30 [-2.90, 2.30]

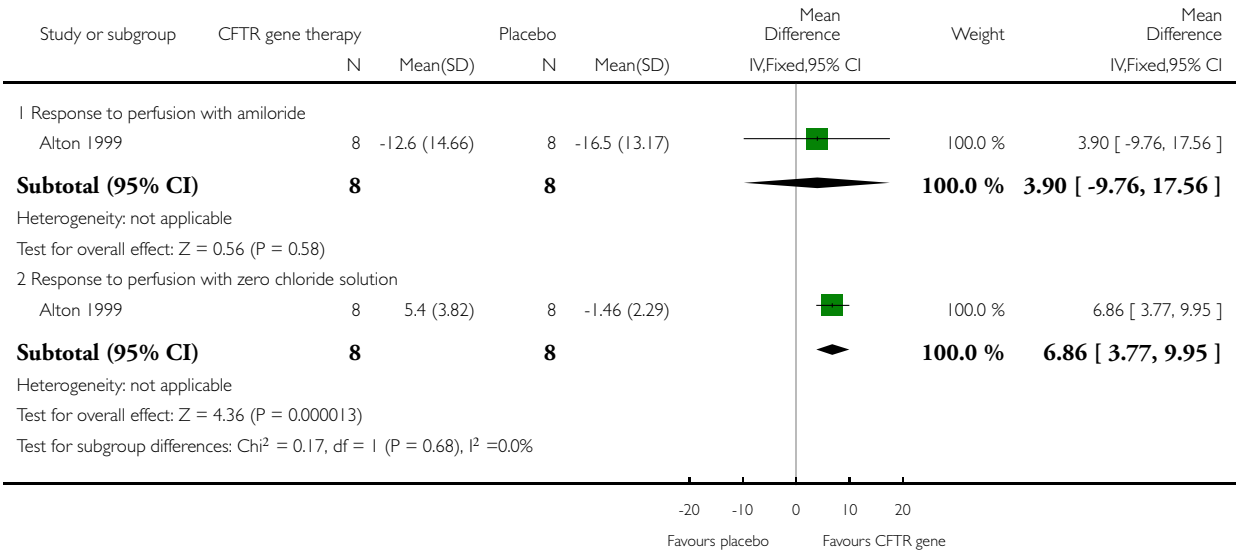


Analysis 1.11. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 11 Lower airway potential difference change from baseline (amiloride and low chloride).

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 11 Lower airway potential difference change from baseline (amiloride and low chloride)



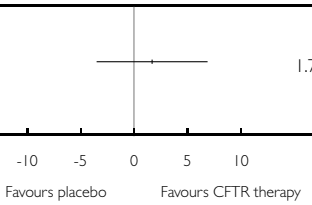
Analysis 1.12. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 12 Lower airway potential difference - post treatment.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 12 Lower airway potential difference - post treatment

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
1 At up to 12 months						
Alton 2015	10	-14 (5.38)	7	-15.7 (5.38)		1.70 [-3.50, 6.90]



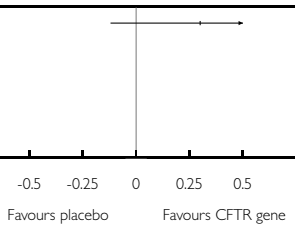
Analysis 1.13. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 13 Measurement of CFTR protein expression (SPQ chloride efflux).

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 13 Measurement of CFTR protein expression (SPQ chloride efflux)

Study or subgroup	CFTR gene therapy N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Alton 1999	6	0.4 (0.49)	7	0.1 (0.22)			0.30 [-0.12, 0.72]
Test for subgroup differences: Not applicable							

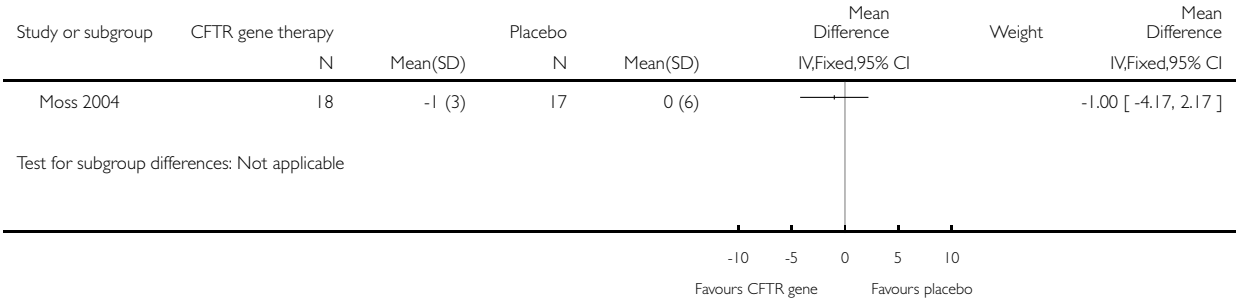


Analysis 1.14. Comparison 1 CFTR gene replacement therapy versus placebo, Outcome 14 Change in validated computerised tomogram (CT) score.

Review: Topical cystic fibrosis transmembrane conductance regulator gene replacement for cystic fibrosis-related lung disease

Comparison: 1 CFTR gene replacement therapy versus placebo

Outcome: 14 Change in validated computerised tomogram (CT) score



ADDITIONAL TABLES

Table 1. Primary outcomes measured

Study name	Respiratory exacerbations	Lung function	Days in hospital	Survival
Alton 1999	Not measured.	Measured, but only at 6 hours post-dose to monitor for adverse effects. Reported in this review.	Not measured.	Not measured.
Alton 2015	Measured respiratory exacerbations requiring any antibiotics (IV or oral) between 11 - 12 months Reported in this review.	Measured relative change in FEV_1 % predicted, change in FVC % predicted, at baseline, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months, Measured KCOc, TLCOc, lung clearance index and $MEF_{25-75\%}$ at 12 months. 1, 2, 3 and 12 month data reported in this review.	Not measured.	Not measured.
Moss 2004	Measured respiratory exacerbations requiring IV antibiotics within 150 days.	Measured FEV_1, FEV_1 % predicted, FVC, at baseline, 30, 60, 90 and 150 days.	Not measured, although number of inpatient episodes within 150 days measured.	Not measured.

Table 1. Primary outcomes measured (*Continued*)

	Reported in this review.	Day 30 and 60 data reported in this review.	Reported in this review.	
Moss 2007	Measured respiratory exacerbations requiring IV antibiotics within 210 days. Reported in this review.	Measured FEV_1, FEV_1 % predicted, FVC, at baseline, 14, 30, 45, 60, 75 and 90 days. Day 30 and 60 data reported in this review.	Not measured.	Not measured.

FEV_1: forced expiratory volume in one second
FVC: forced expiratory volume
IV: intravenous
KCOc: diffusion capacity of the alveolar capillary membrane
$MEF_{25-75\%}$: mid-expiratory flow between 25% and 75% of FVC
TLCOc: transfer factor of the lung for carbon monoxide (TLCOc)

Table 2. Secondary outcomes measured

Study name	Extra treatment	Adverse events	Quality of life	School or work days	Nutrition	New pathogens	Sputum rheology	Mucus clearance	Airway PD
Alton 1999	Not measured.	Measured. Reported in this review.	Not measured.	Not measured.	Not measured.	Not measured.	Not measured.	Only measured saccharine nasal mucociliary clearance, this is not an efficacy measure for CFTR gene replacement therapy to the lung. Not reported.	Measured baseline potential difference, response to perfusion with amiloride, and response to low chloride solution and isoprenaline, measured at baseline and 2 days after study drug. Reported in review.

Table 2. Secondary outcomes measured (*Continued*)

Alton 2015	Not measured.	Measured to 12 months Reported in this review.	Measured at baseline and post treatment (up to 12 months). Reported in this review.	Not measured	Measured at baseline and post treatment (up to12 months). Reported in this review.	Measured *Pseudomonas aeruginosa, Staphylococcus aureus* and *Haemophilus Influenzae* at baseline and post treatment (up to 12 months). Reported in this review.	Not measured.	Measured baseline and post treatment (up to 12 months) 24 hour sputum weight Reported in this review.	Measured baseline potential difference and response to zero chloride solution and isoprenaline measured at baseline and 28 days (+/- 5 days) post treatment (up to 12 months) Reported in this review.
Moss 2004	Not measured.	Measured to 150 days. Reported in this review.	Not measured.	Not measured.	Not measured.	Measured *Pseudomonas aeruginosa* and *Staphylococcus aureus*, (at baseline and Day 90) but data are incomplete. Reported in review.	Not measured.	Not measured.	Not measured.
Moss 2007	Not measured.	Measured to 210 days. Reported in this review.	Not measured.	Not measured.	Not measured.	Not measured.	Not measured.	Not measured.	Not measured.

CFTR: cystic fibrosis transmembrane conductance regulator

APPENDICES

Appendix 1. Search strategy: National Institutes for Health (NIH) Genetic modification Clinical Research Information System (GeMCRIS)

Search dates: 1992 to 20 April 2016
Search term: "cystic fibrosis" (as the medical condition)

WHAT'S NEW

Last assessed as up-to-date: 16 June 2016.

Date	Event	Description
21 July 2016	Amended	Order of authors on byline amended.

HISTORY

Protocol first published: Issue 1, 2006

Review first published: Issue 2, 2007

Date	Event	Description
16 June 2016	New citation required but conclusions have not changed	Despite the addition of a new study to this updated review, our conclusions have not changed. Three new authors have joined the review team. Dr Tim Lee has stepped down as lead author and Professor Kevin Southern has taken this role on
16 June 2016	New search has been performed	A search of the Cystic Fibrosis & Genetic Disorders Review Group's Cystic Fibrosis Trials Register identified eight additional references to the previously listed ongoing study which is now included in the review (Alton 2015).
19 September 2013	New citation required but conclusions have not changed	While a new study has been identified, it is still ongoing and has not yet been assessed for inclusion in this review, hence our conclusions remain the same

(*Continued*)

19 September 2013	New search has been performed	A search of the Cystic Fibrosis & Genetic Disorders Group's Cystic Fibrosis Trials Register identified four references to a single ongoing study which is potentially eligible for inclusion in this review, but will be assessed in full once completed (Alton 2013a)
29 August 2012	New citation required but conclusions have not changed	No new studies were included in this update of the review, so the conclusions remain the same
29 August 2012	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register did not identify any new studies potentially eligible for inclusion in the review
28 September 2011	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register and an additional search of GeMCRIS did not identify any potentially eligible references for this review A study previously listed as ongoing, has now been excluded after confirmation from the investigators that the study did not have a control arm (Alton 2009)
12 August 2010	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register identified one additional reference for possible inclusion in this review (Griesenbach 2008). This abstract described plans for a future clinical trial and has not been included
9 September 2009	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register did not identify any potentially eligible references for inclusion in this review Through personal communication we have identified a new ongoing trial (Alton 2009a)
12 August 2009	Amended	Contact details updated.
11 November 2008	Amended	Converted to new review format.
11 November 2008	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register did not identify any potentially eligible references for this review
20 February 2008	New search has been performed	A search of the Group's Cystic Fibrosis Trials Register identified one new reference (Moss 2007). This is the full paper relating to the abstracts previously included under the study ID Moss 2005. This paper reported full adverse event follow up that had previously been reported as "incomplete", and the new data have been incorporated into the adverse event analyses in this update

(*Continued*)

20 February 2008	Amended	We have replaced the original 'Synopsis' with a new 'Plain language summary' in line with latest guidance from The Cochrane Collaboration
21 February 2007	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

The protocol and review were conceived and drafted by Dr Tim Lee and Professor Kevin Southern. Both authors selected studies and extracted data.

From the update in 2015, three new authors joined the team: Jahan Penny-Dimri and Luke Perry undertook study selection and data extraction and Aisha Aslam undertook analyses and drafted the review update.

Professor Southern acts as guarantor for the review.

DECLARATIONS OF INTEREST

All authors: none known.

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Internal sources

- No sources of support supplied

External sources

- National Institute for Health Research, UK.

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INDEX TERMS

Medical Subject Headings (MeSH)

Cystic Fibrosis [genetics; *therapy]; Cystic Fibrosis Transmembrane Conductance Regulator [*genetics; therapeutic use]; Gene Transfer Techniques; Genetic Therapy [adverse effects; methods]; Liposomes; Randomized Controlled Trials as Topic; Respiratory Function Tests; Targeted Gene Repair [adverse effects; *methods]

MeSH check words

Adolescent; Adult; Female; Humans; Male